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TUBERCLE BACILLI AND ELASTIC TISSUE FIBERS IN THE SPUTA OF DIFFERENT TYPES OF PHTHISIS*

BY ISRAEL RAPPAPORT, M.D., NEW YORK CITY

IN A RECENT publication Ellison and I[1] have described a method for the staining of elastic tissue fibers and tubercle bacilli on the same smear. Speaking of the relationship between these two elements of the phthisical sputum, we concluded that usually the bacilli appear first, then the elastic tissue fibers, when the disease begins; also, that the elastic tissue fibers disappear before the tubercle bacilli, as the process heals.

There were exceptions to this rule which I have observed in my studies of over 200 cases, which we did not include in our report, because we did not consider these observations conclusive enough. I am returning to the subject now, because recent developments seem to give these exceptional observations a significance of which we had little understanding at the time we made them.

A few months ago French clinicians[2] reported series of cases of clinical phthisis in which "abacillary" respectively "prebacillary" stages of the disease were observed. During these stages tubercle bacilli could not be demonstrated in the patients' sputa, not even by cultural or guinea pig inoculation methods.

In the material studied by myself I observed a single case in which elastic tissue fibers were present in the sputum in abundance, while tubercle bacilli could not be demonstrated, not even on repeated homogeneization. Cultural or guinea pig inoculation tests were not done. It was the case of a young woman who came under my observation rather shortly before her death from a Laennec type of multilobar pneumonic phthisis of about six weeks' duration. Unfortunately I could study her sputum only for a short period; I did not therefore consider the observation a conclusive one.

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This case recalled to my mind another similar case which I had observed some years earlier. It was the case of a twenty-three-year-old man who was sent to the mountains with the report of an active right pulmonary process and positive sputum. He was with us hardly a few days, when he became extremely ill with a consolidation of his entire right lung. Though he had a purulent sputum, we could never demonstrate tubercle bacilli in it. At first we thought of a banal pneumonic complication, but the process soon proved to be a rapidly softening phthisical process, to which the patient succumbed in about five or six weeks. During that time we did not succeed in revealing tubercle bacilli in his sputum by the routine staining methods; no cultural or animal inoculation tests were done. I do not know whether elastic tissue fibers were expectorated in this case, as at that time we did not yet have our method for elastic tissue staining.

In the Home for Consumptives, Chestnut Hill, Pa., situated as we were in a metropolitan city, we received a large number of pneumonic phthisis patients, most of whom have been kept previously for some weeks in general hospital wards. The invariable history obtained was—that they were kept as pneumonia cases for weeks, because of negative sputa, and only just before transfer was a positive sputum obtained. These were frequently the very cases in which I found a great discrepancy between the abundance of elastic tissue fibers and the paucity of tubercle bacilli. Very frequently homogeneization had to be resorted to in these cases.

Frankly speaking at the time of these observations I did not attach much significance to these findings. So much so, that in lack of precise records on these cases I could not even establish their number now. However, their number was not large enough to become conspicuous. Like other workers before me, Besancon and Brodiez,[3] Hoesslin,[4] etc., who observed such discrepancy in a few of their cases, I, too, was inclined to blame it on the accidental qualities of the specimen, faulty technic, etc., rather than assume the illogical presence of elastic tissue fibers without tubercle bacilli in the sputa of the cases in question.

Reading now the reports of the French clinicians of their cases of "abacillary phthisis," I was struck with the fact that most of their cases were also pneumonic or bronchopneumonic acute conditions. Just the type of cases in which the absence or paucity of tubercle bacilli at the earliest stages has puzzled me at the time of my studies. I came to the belated conviction that what I have observed in some of these cases was really the appearance of elastic tissue fibers preceding that of tubercle bacilli.

The question of elastic tissue fibers appearing without simultaneous presence of tubercle bacilli in phthisical sputum dates back to Sir Francis Troup, who championed the pathognomonic precedence of elastic tissue fibers over tubercle bacilli almost half a century ago. Just a few years after Koch revealed the tubercle bacillus, Sir Francis Troup published his excellent monograph on the sputum.[5] Here, he described his natural drop method of elastic tissue examination, and urged the search for elastic tissue fibers in all phthisical sputa in the following manner:

"It appears to me that the diagnostic value of curly fiber is apt to be

much underestimated in the present day, when the tubercle bacillus like Aaron's rod swallows up everything else. . . . . In many cases whose symptoms and progress called up initial phthisis into the mind, I have demonstrated elastic tissue where no tubercle bacilli were found, and in one remarkable case I had the opportunity of watching and seeing the advent of the tubercle bacilli months after elastic tissue was first noticed. Since the author became acquainted with Koch's discovery he has carefully examined many hundreds of sputa and has arrived at the conclusion that curly fiber is actually a prodrome, a precursor of the bacillus. . . . In ancient, long-spun-out cases of phthisis, tissue is also to be found pretty constantly though sparingly and in minute morsels, while the bacillus may be missed entirely or for long intervals." Then he goes on relating cases observed by him in which elastic tissue preceded the appearance of the bacilli, and it is quite apparent that the cases were mostly acute pneumonic conditions, similar to those I have above mentioned.

By all appearances recent developments of phthisiology point to the fact that the contentions of Sir Francis Troup are about to be vindicated. It took nearly half a century to come to the appreciation of his unheeded observations. While it is not yet possible to say that his contentions have been proved, evidence is coming forth tending to show that there is a type of phthisis in which elastic tissue fibers are expectorated without tubercle bacilli.

The conception of the French clinicians is that in some phthisical conditions the bacillus passes through a filterable, invisible, ultravirus form before it takes up its usual acid-fast form. This transformation of the tubercle bacillus accounts then for its absence in the so-called pre- or abacillary stages of the disease.

Whether or not we accept the assumption of the French clinicians as regards the ultravirus of the Koch bacillus, our particular question here turns on the point of whether or not in these conditions elastic tissue fibers appear in the sputum before the tubercle bacilli. Information as regards this question is meager, for the simple reason that these conditions are mostly observed in the general wards where investigation of the sputa for elastic tissue fibers is not usually done.

Of similarly great interest is the other contention of Sir Francis Troup, that in chronic cases the disappearance of elastic tissue fibers follows that of the bacilli, and that "minute morsels" of elastic tissue may still be demonstrated when no more bacilli are to be found. In our quoted publication Ellison and I have expressed ourselves in agreement with former workers, stating that as a rule the elastic tissue fibers disappear before the tubercle bacilli. However, in that publication we restricted our report to the two main and most frequent types of elastic tissue elements. We did mention that Besancon and Brodiez described other forms besides, which we have left unconsidered for the sake of simplicity. Besancon and Brodiez described an amorphous form of elastic tissue elements which comes in smaller or larger patches. In the study of my own material I have also observed amorphous forms of elastic tissue elements in single morsels or heaped patches, which I found to be definitely pathognomonic of old cirrhotic phthisis. I found this

form to appear quite frequently even in already long arrested negative sputum cases, in which only Much's granules could be demonstrated but never whole bacilli. Assuming as I do that these are the elements Troup is referring to with his "minute morsels," I think that my observations are bearing out the contention of Troup to the full. Besancon and Brodiez, who emphasize that in their experience elastic tissue always disappeared before the bacilli, also mention the persistence of the amorphous elastic tissue elements after the bacilli have already disappeared, in some of their cases.

Summing up, I must emphasize here—that I fully realize the inconclusiveness of my studies as far as their bearing on the here-discussed questions are concerned. The purpose of this communication is rather to call attention to these most interesting questions, and stimulate investigation in this direction. It seems to me that such investigations should be carried out in the general hospitals, where these atypical pneumonic processes are usually found in the earliest stages. While the relations of disappearance of the elastic tissues and the tubercle bacilli should be studied in the tuberculosis wards, all the more so—because they have, in my opinion, the greatest bearing on prognosis in phthisis.

Another question of great interest is the clinical significance of the relative quantities of elastic tissue fibers as compared with the quantities of tubercle bacilli. It is quite evident that only extremes in the positive as well as negative direction are of any significance. Since elastic tissue expectoration means pulmonary tissue destruction it seemed very logical to me that its persistence, even if not excessive, should speak for progress of the disease rather than persisting bacillus expectoration, which may only signifiy an open lesion without spread. Careful investigations of this question with my material, with serial x-ray control and comparison of parallel cases, led me to the following preliminary conclusions. Excessive amount of elastic tissue expectoration invariably means rapid softening and caseation of extensive lesions, or cavitation of confluent proliferative lesions of a more chronic character.

My first thought was to identify excessive elastic tissue expectoration with extensive, now designated as exudative, caseous processes. My observations have not borne out this assumption. Widespread genuinely proliferative lesions lead to vast amount of elastic tissue expectoration just as frequently as do exudative lesions, and may be even more so. I have observed this in some chronic proliferative cases over considerable periods, frequently just before extensive fibrosis began and the process took a favorable turn. The most remarkable thing about these cases was—that the patients had no clinical signs of activity or such signs were just abating, while elastic tissue fibers continued to stream out in great abundance. On the other hand I have observed cases of the exudative type in which a sudden excessive expectoration of elastic tissue fibers coinciding with clinically very active periods, enabled me to foretell coming on hemorrhages. In fact, I lost two patients in fulminant pulmonary hemorrhages just after the appearance of such excessive elastic tissue expectoration. It would seem therefore that the amount of elastic

tissue expectorated must also be judged in the light of other clinical phenomena in each individual case.

I believe, however, that persistent elastic tissue expectoration, in cases with or without mild symptoms of clinical activity, has greater significance than the mere expectoration of bacilli has. I found that such cases progressed to slow cavitation, the course of which was chronic but definitely destructive in the long run. The longer such elastic tissue expectoration lasted the lesser were the chances of the process going to spontaneous healing. On the other hand, in cases showing chronic low grade activity or even moderate degrees of activity in which the sputa showed gradual disappearance of elastic tissue fibers, even if bacillus expectoration continued undiminished, the x-rays soon showed definite signs of absorption, and with more or less delay these cases came to clinical arrest. In one word periodical check-up of the elastic tissue expectoration permitted far-reaching prognostic predications which were borne out by the events to a remarkable degree.

After a while, these studies of the elastic tissue behavior in the different stages of the various conditions enabled us to compare types and stages of phthisical conditions from the standpoint of elastic tissue findings. Frequently we were able to make even a diagnosis of the particular phase or type of the disease from the sputum examination alone, as supported by the clinical history of the case in question.

It is my firm conviction that large scale investigation with a standard of counting and comparing expectorated quantities of elastic tissue elements, as observed and checked up periodically, would reveal such regularities typical to certain types and phases of phthisis, as to permit definite diagnosis and prognosis in a large number of cases. It would also enable us to set up such type and phase classifications of phthisis that would be of more value from a practical point of view, than the usually accepted divisions of today.

REFERENCES

1. Rappaport, and Ellison: J. Lab. & Clin. Med. **14**: 261, 1928.
2. Durand, et al.: Compt. Rend. Soc. de biol. **99**: 30 and 32, 1928.
3. Besancon, and Brodiez: Rev. de la Tuberc. **3**: 398, 1922.
4. Hoesslin, H.: Das Sputum, Berlin, 1926, J. Springer.
5. Sir Troup, Francis: The Sputum, Edinburgh, 1886, Oliver & Boyd.

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INCIDENCE OF BACTERIA IN SPUTUM CULTURES FROM FIFTY PATIENTS HAVING ASTHMATIC BRONCHITIS*

A Preliminary Report

By Margaret J. McKinney, M.S., Chicago, Ill.

I

IN THE preparation of sputum vaccines used in the treatment of certain asthma patients, there occurs the opportunity of studying the type of bacteria resulting from sputum cultures. The sputums studied were obtained from 50 patients having asthma, who were not sensitive to proteins used in routine skin testing, and whose history showed the original asthmatic attack to have followed bronchitis or a "cold."

II

Koessler and Moody[1] in 1917 made vaccines from both aerobic and anaerobic cultures of sputum from patients having bronchial asthma, and Huber and Koessler[2] in 1922 mention such vaccines in treatment of a case of bronchial asthma. The organisms obtained by them from aerobic cultures were pneumococci, streptococci, the influenza bacillus, and M. catarrhalis. Rackemann[3] in 1920 reported the growth, from sputum cultures from asthma patients having bacterial infections of the bronchi, of nonhemolytic and hemolytic streptococci, Staphylococcus albus and aureus, and gram negative bacillus and a gram negative coccus. Rackemann and Graham[4] in 1924 reported further work on vaccines from asthma sputums, with growth of organisms occurring in the following importance, green streptococci, nonhemolytic streptococci, staphylococci, pneumococci, B. influenzae, and a gram-negative bacillus. Rackemann and Scully[5] in 1928 found again that green and hemolytic streptococci, and staphylococci seemed to predominate in asthma sputum, with growth also of pneumococci and B. influenzae. Thomas, Famulener and Touart[6] in 1924 identified organisms from asthma sputum cultures, finding a predominance of streptococci, green, hemolytic and indefinite, in the order named, with staphylococci second in number. Other organisms recovered were atypical gram-negative cocci and bacilli, pneumococci, M. catarrhalis, enterococcus, B. coli communis, B. fecalis alcaligenes, and Friedlander's bacillus. Wherry[7] in 1927 identified the organisms from asthma as M. aureus, M. albus, M. flavus, M. catarrhalis, Streptococcus anhemolyticus, Pneumococcus I, II, III, and IV, B. influenzae, B. mucosus (Friedlander's), B. duplex liquefaciens, and B. abortus. Walker and Adkinson[8] (1928), in a report covering several years of work on sputum from asthmatic bronchitis, record the predominance of streptococci, differentiating the strains, with staphylococci following in importance, other bacteria not being classified.

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Noble, Fisher and Brainerd[9] in a recent article on acute respiratory infections, have made a study of aerobic flora from both normal and infected respiratory tracts. They find that the normal respiratory flora varies with the subject and the time of culture. However, the average basic flora consists of green streptococci and gram-negative cocci. Frequent transient organisms are streptococci, B. influenzae, staphylococci, and diphtheroids. Occasional transients are hemolytic streptococci, M. catarrhalis, and Friedlander's bacillus.

III

The technic of making the sputum cultures was as follows: Sputum raised by the patient during an asthmatic attack was collected in a sterile container and taken as soon as possible to the laboratory. Using a looped inoculating needle, portions of the sputum were washed by gently shaking the inoculated loop in sterile physiologic salt solution. The washed loopsful of sputum were then streaked in sunburst pattern on blood agar plates (5 per cent defibrinated human blood) and were also inoculated into tubes of plain and ascitic fluid broth. After incubation for twenty-four hours at 37° C. the cultures were examined. Colonies were picked from the plates for subculture on other blood agar plates, broth cultures were streaked on blood agar plates, and these cultures were examined and subcultured after suitable incubation. Pure cultures were transferred to various sugar media for identification of the organisms. Vaccines were made by washing pure cultures on plain agar slants or in plain broth with sterile physiologic salt solution, counting by comparison with a known number of red blood cells, diluting to the desired number of organisms per cubic centimeter, and heating in the water-bath at 56° C. until all organisms were killed.

Streptococci were cultured in broth after their character on blood agar was determined. They have been classed here only as hemolytic and nonhemolytic, the green-producing strains being included in the nonhemolytic group. Further identification of streptococci is reserved for a later discussion.

Many gram-positive diplococci were recovered and were tested for bile solubility. No organisms having all the characters of the pneumococci were cultured.

Organisms of the staphylococcus, neisseria, Mucosus capsulatus, and diphtheroid groups grew well from initial cultures on blood agar. Other organisms mentioned were obtained also from initial plate culture. No attempt was made to isolate the influenza bacillus by means of special media.

IV

Table I shows the type and number of organisms recovered from sputum cultures. Streptococci, chiefly nonhemolytic, predominated in culture. The neisseria group, gram-positive diplococci (bile insoluble), and staphylococci followed in the order named. Other organisms recovered were the Mucosus capsulatus group (including Bact. pneumoniae), diphtheroids, gram-positive cocci (as M. tetragenous), yeast-like organisms, Bacillus subtilis, streptothrix, and a vibrio.

TABLE I

INCIDENCE OF BACTERIA IN FIFTY ASTHMATIC SPUTUMS

ORGANISMS CULTURED		NUMBER	TOTAL NUMBER	PER CENT
Streptococci	Nonhemolytic	38	43	23.62
	Hemolytic	5		
Neisseria	N. crassus	2	32	17.58
	N. sicci	2		
	N. catarrhalis	1		
	Others	27		
Gram positive diplococci (bile insol.)			27	14.83
Staphylococci	Staph. albus	19	23	12.63
	Staph. aureus	4		
Mucosus capsulatus group	Bact. pneumoniae	4	14	7.69
	Others	10		
Diphtheroids	C. Hoagii	2	14	7.69
	C. xerosis	1		
	C. flavidus	1		
	C. Hoffmanii	1		
	Others	9		
Gram + cocci	M. tetragenous	2	8	4.39
	Others	6		
Yeast-like organisms			9	4.94
Bacillus subtilis			8	4.39
Streptothrix			3	1.64
Vibrio group			1	0.54
Total number of organisms cultured			182	99.94

V

The results of this study are in general accord with previous investigations on asthma sputums in finding a predominance of streptococci in culture. These results are slightly different, however, in placing the gram-negative cocci (neisseria group) second in number, and the staphylococci fourth. How many of the organisms recovered belonged to the normal mouth flora of the patients was not determined.

REFERENCES

1. Koessler, K. K., and Moody, A. M.: Trans. Chi. Soc. Int. Med. Nos. 1 and 2, 7, 1917.
2. Huber, H. L., and Koessler, K. K: Arch. Int. Med. **30:** 690-760, 1922.
3. Rackemann, F. M.: J. Immunol. **4:** 373, 1920.
4. Rackemann, F. M., and Graham, L. B.: Arch. Int. Med. **34:** 295, 1924.
5. Rackemann, F. M., and Scully, M. A.: New England M. J. **199:** 314, 1928.
6. Thomas, W. S., Famulener, L. W., and Touart, M. D.: Arch. Int. Med. **24:** 85, 1924.
7. Wherry, W. B.: J. Infect. Dis. **41:** 177, 1927.
8. Walker, I. C., and Adkinson, J.: Arch. Int. Med. **41:** 601, 1928.
9. Noble, W. C., Jr., Fisher, E. A., and Brainerd, D. H.: J. Prevent. Med. **2:** 103, 1928.

BASAL UREA ELIMINATION

By Dr. Charles Richet, Jr.,* Paris, France

IN A DOZEN contributions published during the last three years I have presented my studies of a new conception which in French I have termed *azoturie basale*.[1] In English the expression basal urea elimination seems to express the same idea. This concept in its own field is analogous to that of basal metabolism as presented by du Bois.

It is well known that the basal metabolism represents the heat produced per square meter body surface per hour with the individual at rest, at an optimal temperature and fasting for at least twelve hours.

I have formulated a similar definition of basal urea elimination. *Basal urea elimination is measured in terms of the elimination of urea per square meter body surface over a period of twenty-four hours with the individual in an ideal state of nitrogen equilibrium.*

Body surface is measured by du Bois' formula for men and women or by Rubner's formula for dogs. We have measured the urea elimination rather than the nitrogen elimination for many reasons. The former determination is much simpler in medical practice. The relation between the urea nitrogen and the total nitrogen elimination in urine is always constant.† With subjects in good health and in nitrogen starvation we neglect the 5 per cent nitrogen elimination in the stools.

The chief consideration in the determination of basal urea elimination is dietary. In our experiments dogs have been put successively on the following diets: starvation, sugar diet, fat diet, and sugar and fat diet. From these studies we found that urea elimination is minimal on a combined fat and sugar diet (six grams of sugar and six grams of fat per kilogram body weight). Dogs which could not tolerate this diet without diarrhea were eliminated. As a rule the urea elimination became stabilized by the ninth or tenth day, after which it remained constant. We found that with dogs on this diet the urea elimination per kilogram was variable; but when we expressed it in relationship to square meters of body surface, we found that the elimination became regular.

The urea elimination per square meter is always about the same (about 7.65 grams).

In our bibliographic research we found that Irwin Voit's results were comparable. This author found that three dogs of 29, 18 and 7 kilograms weight respectively, showed a nitrogen equilibrium during starvation of 5.2, 6.6 and 5.2 grams per square meter per twenty-four hours.

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†It is lower in nitrogen starvation than on the usual diet. I found it reduced on the average 70% in man and 75% in the dogs.

TABLE I

	ELIMINATION OF UREA IN GRAMS PER SQUARE METER PER 24 HOURS	ELIMINATION OF UREA IN GRAMS PER KILO PER 24 HOURS
Dogs weighing more than ten kilograms (4 dogs)	7.6	0.33
Dogs weighing nine kilograms (4 dogs)	8.4	0.44
Dogs weighing from seven to nine kilograms (10 dogs)	7.4	0.41
Dogs weighing less than seven kilograms (6 dogs)	7.5	0.46

In view of these findings I have formulated the following law: *In nitrogen equilibrium the urea elimination is proportional to the surface and not to body weight.*

But for man it was impossible to give such an alimentation quite free from protein, limited entirely to sugar, starch and fat. We lost hope of applying these experiments to man when unexpectedly we made the following verification:

If a man has in his dietary a sufficient number of calories (about three thousand per day), nitrogen equilibrium is the same whether his intake consists of eight, ten or fourteen grams of protein. Nitrogen elimination in any of these cases corresponds to the carburation of nineteen grams of protein (wear and tear quota of Rubner). If it is impossible to feed a man for ten or twelve days without protein, it is not difficult to provide him with a diet which he can tolerate and which contains only twelve to fourteen grams of protein.* This we did on ten adults and twelve children whom we put on such a diet; it enabled us to obtain satisfactory results.

The following standard dietary was given: sugar, starch, all kinds of fruit, nuts (except chestnuts, walnuts, hazelnuts and almonds), jam, potatoes in moderation as they contain 2 per cent protein, carrots, butter, oil, Devonshire cream.

The major portion of the starch is contained in a special bread. Starch (100 grams) is mixed with butter (60 grams) and sugar (90 grams). Just enough water is added to dissolve the sugar. The mixture is raised with a little yeast for twenty-four hours to make it light. It is baked in a hot oven. This bread is highly nutritious, and one has no difficulty in eating 200 grams a day. Its caloric value is about 500 calories per 100 grams.

On this diet, nitrogen equilibrium is established by from the seventh to the tenth day. For the next three days the urea elimination is measured. With this method we have found that the elimination of urea per square meter per day for an adult averages 2.5 grams.

Individual differences may be of importance. Figures for the normal may range between 1.9 grams and 3.1 grams or roughly between 2 and 3 grams per square meter per twenty-four hours. For instance the first three persons studied were three adult doctors in good health and of great physical activity.

*With the food tables appended at the end of Lusk's book "The Science of Nutrition," it is easy to arrange a diet containing more than 2,500 calories and less than 14 grams of protein.

Their food contained on the average 13.4 grams, 13.9 grams, 14.1 grams of protein per day, and the basal urea elimination was respectively 2.8 grams, 2.53 grams and 2.04 grams. We must admit then that the number 2.5 grams per square meter per day may vary as much as 20 per cent in either direction. The individual differences in this determination appear to be greater than the normal variation in basal metabolism determination, where they do not exceed 10 per cent.

Individual variations are greater than class differences, such as the variation between men and women (2.41 and 2.91); between people past the age of forty-five years and persons from ages twenty-eight to forty-five years (2.39 and 2.74); between workers and those who are at rest in bed (2.46 and 2.56); between those who eat ten grams or less of protein per twenty-four hours and

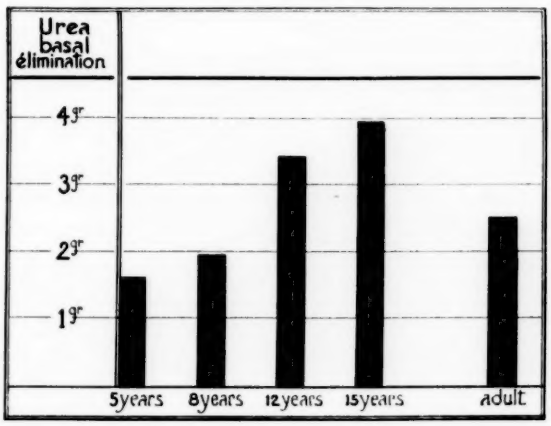

Chart I.—This schema shows the variations of urea basal elimination at different ages. We see that it increases from five to fifteen years of age and that afterward it decreases.

those who eat between ten and fourteen grams (2.39 and 2.81); and between those who weigh either more or less than sixty kilograms (2.56 and 2.46).

For children the basal urea elimination is not the same as for adults, as is shown in Table II.

TABLE II

AGE OF THE CHILDREN	BASAL UREA ELIMINATION
From 4 to 6 years (4 children)	1.65
From 7 to 9 years (4 children)	1.97
From 10 to 12 years (2 children)	3.46
From 13 to 14 years (2 children)	3.95

I conclude from these figures that children have a basal urea elimination which varies with increasing age. Slight in the first years, it increases suddenly at about the tenth year and seems to be maximal toward the fifteenth year. The accompanying graphic chart shows this phenomenon well.

What is the biologic value of basal urea elimination? The basal urea elimination represents the elimination of urea per square meter area with a protein diet insufficient to cover the needs of the organism, but containing a

sufficient number of calories for maintenance. On such a diet instead of having an excess consumption of protein, one has a dearth consumption. As a consequence the organism will burn a portion of its own protein in order to provide maintenance of life. Since the calorie needs are covered, the body will burn just the quantity necessary for maintenance.

The measurement of this nitrogen elimination records quantitatively the wear and tear or better the cellular needs of the organism.

CONCLUSION

The basal urea elimination is the quantity of urea eliminated per day per square meter body surface in subjects on a diet providing ample caloric value but insufficient in protein.

This amounts in dogs to 7.65 grams; in human adults 2.50 grams, varying between 2 and 3 grams. In children it is about 1.65 grams at five years of age, 2 grams at eight years, 3.4 grams at eleven years, and 4 grams at fourteen years, with important individual variations.

The study of the basal urea elimination seems to us to offer promise of distinct value in studies in physiology, in which domain I have been carrying on my investigations, and in pathology, where it is still, I think, a no man's land.

REFERENCES

1. Richet, Charles: l'Azoturie et l'Azotémie Basales, Arch. Internat. Physiol. **27**: 334, 1926.
2. Richet, Charles, and Monceaux, R.: Les Regimes Hypoazotés, Presse méd. **55**: 865, 1926.

THE URIC ACID INCREASE IN THE BLOOD OF PATIENTS WITH CARDIAC DECOMPENSATION

By J. Lisle Williams, M.D., Chicago, Ill.

THE uric acid content of the blood is increased in many diseases. Most of the studies have been made on such diseases as gout, nephritis, hypertension, arteriosclerosis, starvation, epilepsy or acute intoxications in which the kidneys have been damaged and the increased blood uric acid could be explained on the basis of deficient elimination.

In a previous communication[1] it was pointed out that in the blood of patients with chronic nephritis and hypertension and with myocardial insufficiency there was a much higher concentration of uric acid than in those whose cardiac function was adequate. It was also stated that with clinical improvement there was a diminution in the concentration of uric acid in the blood. Other than this report no systematic study of the blood uric acid of patients with heart disease but without nephritis is to be found in the literature.

This study was made on patients in varying stages of cardiac decompensation but without nephritis as determined by careful clinical study, functional tests, frequent and repeated examinations of the urine and in several instances by postmortem examinations. During the past six years 22 patients were selected and studied. There are 14 males and 8 females in the group. The ages varied from eight to seventy years and the average was about forty years.

The clinical diagnosis was chronic myocarditis with 8 patients, 6 of whom had hypertension. Thirteen had chronic valvular disease of the heart. Of these, 8 had mitral stenosis either alone or combined with mitral regurgitation. In 4 patients there was aortic regurgitation and in one both aortic stenosis and regurgitation. Mitral regurgitation was the sole lesion detected in one patient. In another, syphilitic aortitis with mitral regurgitation was the diagnosis.

The blood uric acid was determined in all patients and with several, repeated determinations (five in one instance) were made. The blood urea nitrogen, nonprotein nitrogen and creatinine were also estimated in almost every case. The carbon dioxide combining power of the plasma was measured in several patients and the amino acid nitrogen of the blood of a few was also recorded.

The blood uric acid varied from 3.0 to 9.8 mg. in the maximum range of all patients and all determinations but the averages for these patients ranged from 3.6 to 6.9 mg. with a grand average of 4.75 mg. per 100 c.c. of blood.

The averages for blood urea nitrogen were 9.4 to 45.7 mg. with a final average of 20.8 mg. per 100 c.c. For nonprotein nitrogen these values were 27.4 to 66.7 and 40.1 mg. as a grand average. For creatinine the range was

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1.1 to 2.1 mg. with 1.5 mg. for the mean. The figures for plasma bicarbonate (6 patients) were 53.9 to 73.6 and an average of 59.2 vol. per cent. The amino acid nitrogen was determined in 15 patients and the mean values ranged from 6.0 to 8.7 mg. with 7.4 mg. as the average.

The average maximum and minimum and the grand averages are summarized in Table I.

TABLE I

AVERAGES*	URIC ACID	UREA NITROGEN	TOTAL N. P. N.	CREATININE	AMINO ACID NITROGEN	CO_2 COMB. POWER
Maximum	6.9	45.7	16.7	2.1	8.7	73.6
Minimum	3.6	9.4	27.4	1.1	6.0	53.9
Mean	4.75	20.8	40.1	1.5	7.4	59.2

*Uric acid, urea nitrogen, total nonprotein nitrogen, creatinine and amino acid nitrogen in mg. per 100 cc.: CO_2 combining power in vol. per cent.

For the determination of the various nitrogenous constituents the methods of Folin and Wu,[2] and of Folin[3] were used except that for blood urea nitrogen Van Slyke and Cullen's[4] modification of Marshall's urease method was used. For uric acid the silver lactate precipitation method and Folin's[5] later modification were used. The direct method was found to yield results 0.2 to 0.5 mg. higher than those reported. For the estimation of the alkali reserve Van Slyke's method was used.

The systolic blood pressure varied from 88 to 240 mm., the average of the maximum blood pressure recorded being 157 mm. of mercury, the average of the minimum being 138 mm. The diastolic pressure varied from 50 to 140 mm. of mercury.

The phenolsulphonephthalein test was done on several patients and varied from 50 to 65 per cent for two hours.

The urine was examined repeatedly both during the stage of decompensation and also during improvement. Albumin was present in all but three at some time during the period of decompensation but was absent in all for at least one test during the periods of recovery or improved compensation. Likewise in many patients casts or red blood cells were detected microscopically in the urine sediment during the time when cardiac decompensation was evident.

The Wassermann test was made on the blood of all the patients and was positive in four, all of whom gave other clinical evidence of syphilis.

Dyspnea was the one symptom present in all the patients and was the chief symptom in 19 patients. Orthopnea was marked in 7 patients. Cough was prominent in 14 patients. Six patients complained of palpitation of the heart and in 5 precordial pain or distress was mentioned. Dizziness was present in 4 patients and weakness was pronounced in 5.

Enlargement of the heart was the most important physical abnormality noted and was present in all but one, the enlargement however being only slight with 4 patients. Edema of the lower extremities and trunk was quite marked in 13 patients and was present at times in several others.

Enlargement of the liver was demonstrable in 20 patients. In the 2 patients in whom the liver was not palpable, the blood uric acid was the lowest of the entire series, the values being 3.3 and 3.7 mg. per 100. c.c.

Signs of pulmonary hypostasis or hydrothorax were definite in 14 patients at some time during the clinical course studied.

Of the 22 patients, 10 were known to be dead after a period from one month to four years. Only 4 were known to be improved, 3 were in worse condition when last heard from, 3 were unchanged and 2 could not be followed after a few months.

Of the 10 patients known to be dead all had clinical evidence of a failing heart as the terminal picture. One had an associated pneumonia. Postmortem examination of 5 disclosed as the cause of death, heart disease with associated passive hyperemia of the lungs, liver and kidneys. Microscopic examination of the kidneys revealed changes associated with passive congestion but no appreciable nephritis was found.

Nine patients were studied during the periods of marked cardiac decompensation and also during remissions when definite improvement was noted. The averages are grouped in Tables II and III.

TABLE II

PATIENTS WITH MARKED DECOMPENSATION

MG. PER 100 C.C.	UREA N.	N. P. N.	URIC ACID	CREATININE	AMINO ACID N.
1	23.6	43.7	5.2	1.9	
2	19.6	39.8	6.1	2.4	
3	38.3	59.9	7.3	2.3	
4	28.4	40.7	5.3		
5	75.5	94.5	9.4	2.3	
7	21.0	41.2	4.8	1.9	
8	18.3	38.4	6.3	1.6	7.5
10	21.8	47.5	4.5	1.4	8.5
22	19.5	36.0	4.1	1.5	6.7
Average	29.6	49.0	5.9	1.7	7.7

TABLE III

PATIENTS DURING IMPROVEMENT

MG. PER 100 C.C.	UREA N.	N. P. N.	URIC ACID	CREATININE	AMINO ACID N.
1	22.5	38.9	4.6	1.8	
2	15.2	34.1	3.7	2.5	
3	28.6	47.1	6.4	1.9	
4	13.8	31.3	3.1		
5	15.8	34.7	3.3		
7	17.1	39.4	3.6	1.6	
8	19.8	41.4	5.2	1.4	
10	22.9	42.4	4.2	1.2	7.5
22	23.3	37.3	3.0	1.6	6.6
Average	19.9	38.5	4.1	1.7	7.1

From these averages it is apparent that during improvement there is a decreased concentration of all the nitrogenous waste products of the blood except creatinine. The average decrease in blood urea nitrogen was 9.7 mg., of nonprotein nitrogen it was 10.5 mg., and of uric acid it was 1.8 mg. per 100 c.c., but whereas the relative percentage decrease in nonprotein nitrogen was 22 per cent and of urea nitrogen 32 per cent that of the uric acid was 44 per cent and this in spite of the well-known fact that uric acid is the most difficult of

all these substances to be excreted by the kidneys. That other factors than a mere improvement in the circulation of the kidneys, are involved, seems likely.

With the evident improvement of the cardiac decompensation in these 9 patients there was also a marked diminution in edema and in the size of the liver. With some patients the liver margin receded several centimeters as compensation was regained and concomitant with this change there was a considerable decrease in the amount of circulating uric acid; also when heart failure again supervened and death approached, the opposite condition again prevailed. The amino acid nitrogen of the blood was studied in 3 patients and in two of these it was decreased in its concentration.

In diseases of the liver in which there is a considerable destruction of liver tissue, such as eclampsia, acute yellow atrophy and hyperemesis gravidarum, there is a marked increase in the concentration of uric acid in the blood. That the liver has something to do with uric acid metabolism is accepted by most workers in this field. Folin and associates[6] found in the dog that an Eck fistula is without effect upon the destruction of uric acid intravenously injected. However in liver extirpation experiments as recorded by Perroncito,[7] and Bollmann, Mann, and Magath,[8] the level of circulating uric acid arises rapidly when this organ has been removed in spite of the presence of intact kidneys. Thus it might appear reasonable to ascribe some of the retained uric acid in the blood of patients with marked cardiac insufficiency to the impairment of the function of a liver, partially incapacitated by extreme venous engorgement.

The diagnostic value of the quantitative analysis of the blood for various nonprotein nitrogen extractives is well recognized in nephritis. That relatively high levels of these substances may be present in the circulating blood of patients without nephritis is evident from a study of Table II, where No. 5 has a nonprotein nitrogen of 94.5 mg. per 100 c.c. The absence of nephritis in this patient was verified by postmortem examination. Careful clinical study and repeated chemical analyses of the blood had previously demonstrated normal functioning kidneys when cardiac compensation was restored. On one such occasion the blood uric acid was 2.2 mg. per 100 c.c. The other nitrogenous waste products were normal in amount and the urine was free from albumin and casts.

In 16 of the 22 patients normal values for the various nitrogenous substances other than acid were obtained on at least one occasion. In 5 of the 6 others the values were only slightly elevated and with one a moderate increase was demonstrated at some one period of study.

CONCLUSIONS

1. Cardiac decompensation without nephritis is associated with a marked increase in the concentration of blood uric acid and a moderate increase in urea and nonprotein nitrogen.

2. There is a marked decrease in the concentration of uric acid and a moderate decrease in urea and nonprotein nitrogen of the blood following improvement in cardiac efficiency.

3. This decrease is accompanied by a diminution in the size of the liver and by an improvement in edema.

4. The results cited here support the hypothesis that the liver is an important organ in uric acid metabolism.

5. The determination of the blood uric acid together with the other nonprotein nitrogen extractives of the blood is of diagnostic value only when combined with careful clinical study and repeated uranalyses.

REFERENCES

1. Williams, J. Lisle: The Total Nonprotein Nitrogen Constituents of the Blood in Chronic Nephritis With Hypertension, Arch. Int. Med. **28:** 426-433, 1921.
2. Folin, O., and Wu, J.: A System of Blood Analysis, J. Biol. Chem. **38:** 81, 1919.
3. Folin, O.: A New Colorimetric Method for the Determination of Amino Acid Nitrogen in Blood, J. Biol. Chem. **51:** 377-391, 1922.
4. Van Slyke, D.D., and Cullen, G. E.: J. A. M. A. **62:** 1558, 1914.
5. Folin, O.: A Revision of the Method for Determining Uric Acid, J. Biol. Chem. **54:** 153-170, 1922.
6. Folin, O., Berglund, H., and Derick, C.: The Uric Acid Problem, J. Biol. Chem. **60:** 361-371, 1924.
7. Perroncito: Reforma med. 1921, quoted by Feinblatt, H. K.: Uricacidemia, Arch. Int. Med. **31:** 758-765, 1923.
8. Bollmann, Jesse L., Mann, F. C., and Magath, T. B.: Uric Acid Following Total Removal of the Liver, Am. J. Physiol. **72:** 629-646, 1925.

EXPERIMENTAL ELIMINATION OF ADHESIONS CAUSED BY INTRAPERITONEAL INJECTION OF NEOARSPHENAMINE*

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THE present report is based on the autopsy results from 67 animals injected intraperitoneally, two to six times, with solutions containing one per cent neoarsphenamine.

LITERATURE

Favorable clinical results in infants are reported in 25 out of 28 cases.[1, 2, 3, 4] The incidence of adhesion formation in infants, however, is not yet established since there are only three reported autopsies.[2, 4] One infant showed no adhesions following six injections; one was free from adhesions following only 2 injections; and the third died from peritonitis following an accidental injection into the rectus sheath. A clinical search for adhesions was reported in 2 cases,[3] but the negative results have little weight, since surgeons agree[5] that abdominal adhesions rarely cause symptoms although they may eventually produce chronic pain or even acute intestinal obstruction, and since only those adhesions which distort the stomach or colon are commonly diagnosed by the x-ray.[6]

The animal experiments reported to date include only results in rabbits and the early, incomplete results of the present series.[2, 4] Rosenberg's results[1] from 35 rabbits include a temporary chemical peritonitis, perihepatitis, and perisplenitis; some manifestations of discomfort for a few hours; and, "in rare instances" adhesion formation. Yampolsky and Klugh[3] reported that rabbits receiving 10 injections of neoarsphenamine were "still alive and healthy," while "some of those" receiving alternate injections of neoarsphenamine and mercurosal died and showed no adhesions.

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A knowledge of the present conception of the physiology of absorption from the peritoneal cavity, of the pathogenesis and prevention of adhesions, and of the pharmacology of neoarsphenamine was, of course, fundamental to the present study. Cunningham,[6] Hertzler,[5, 7] Voegtlin,[8] and others,[9, 10] have recently written excellent critical reviews of the literature in these fields.

EXPERIMENTS

Our initial quest was finding the species most subject to the formation of adhesions after intraperitoneal injections of neoarsphenamine. The procedure used throughout these experiments included: taking full sterile precautions in preparing solutions and in making injections; basing dosage on 0.012 gm. of neoarsphenamine per kilogram bodyweight in all animals (first injection was always three-fourths of this dose); using in practically all cases a one per cent strength and a seven day interval; securing numerous controls by using the contents of a single ampoule of neoarsphenamine in animals from several different experiments; noting carefully the animal's reactions, health, and stools; and performing complete autopsies after a varying number of injections and at varying time intervals after the last injection. The temporary inflammatory changes found were similar but less severe than those of Rosenberg,[1] and are ignored in this report because they were influenced by the variation in time between the last injection and the autopsy. Since single injections did not produce permanent changes, they are also omitted. Variations in the degree of histologic changes in the extensive and varied peritoneal surfaces were found very difficult to compare: accordingly the experiments were gradually judged more and more on the basis of adhesion formation. This seemed to be the only permanent macroscopic change. The causes of death other than experimental or pneumonia (incidence about the same as in animals used in other experiments) are also mentioned.

Eight guinea pigs, 10 rabbits, and 6 white rats, injected 3 to 6 times each, showed no formation of adhesions. The only pathology found was thickening and engorgement of the peritoneum about the site of injection in one rabbit. The "rare instances" of formation of adhesions in rabbits reported by Rosenberg[1] may have been due to a larger series, to a stronger (2 per cent) solution or, possibly, to a more irritant lot of neoarsphenamine.

In sharp contrast to the above results, 6 out of 7 dogs showed the formation of adhesions. Four of these dogs had definite but harmless adhesions following 2 to 6 injections when autopsied fourteen to thirty-seven days after the first injection. Dog 31 was kept seventy-five days and had no adhesions. The more interesting changes in the other two included:

Dog 27: four injections, twenty-three days (between first injection and death), four adhesions of the omentum to itself formed masses 1 by 1 by 0.5, 3.5 by 1 by 1, 5 by 0.5 by 0.5, and 2.5 by 1 by 0.5 cm., respectively, all containing some fibrous tissue, the largest being engorged with blood, four adhesions between the intestinal serosa, the mesentery, and the omentum, and the ilium was caught in a short loop by a firm adhesion in such a way that an obstruction might easily have occurred;

Dog 30: six injections, thirty-one days, extensive adhesions matted all the peritoneal organs together, a condition which was gradually starving the

dog to death, this was probably due to the injection of the irritant neoarsphenamine solution when there was a paralytic ileus caused by an assistant's attempt to withdraw spinal fluid from the fourth ventricle; bacteriologic cultures were negative.

After the completion of the other experiments here reported, an opportunity arose to do a few experiments on cats. Adhesions were caused in six of the 7 cats injected, though in only two were the adhesions at all marked, and in none were they so situated as to impair health. Cat 64 had 10 c.c. of sterile, slightly cloudly fluid in its peritoneal cavity and evidence of recent extensive fibrinous adhesions of the omentum to the spleen. These findings coincide with Hertzler's view[5] that only temporary adhesions are formed when an irritant injures the mesothelial cells and causes a large exudation, while permanent adhesions result from the destruction of mesothelial cells in the absence of an inflammatory reaction. That most of the adhesions found were of the temporary type is further suggested by Dog 31 where the elapsing of forty days since the last injection may have been a large factor in the absence of adhesions. Temporary adhesions, however, may become permanent by the growth of fibroblasts into the fibrin.[9]

The death of Cat 65 from secondary anemia caused by continual oozing into an extraperitoneal hematoma, 10 by 3.5 by 1.5 cm., was probably due to an accidental injection of the neoarsphenamine into the abdominal wall. This confirms the danger from faulty technic shown by the death of an infant from a similar cause.[4] A small, harmless, subserous hematoma found in Cat 66 was the only indication in the entire series that the sharp needle point had directly injured the intestine. It is interesting to note that the same operator had already made some 320 uneventful intraperitoneal injections into animals before these two accidents occurred. Cats, however, were much more difficult to hold during the injections and would often squirm suddenly when they were apparently tightly and comfortably held.

The sharp contrast between the formation of adhesions in dogs and cats and the complete absence of adhesions in the other laboratory animals is probably due to differences in the omentum which was involved in three-fourths of the adhesions. The guinea pig has very little omentum. In the rabbit, the body of the omentum is insignificant, and it cannot reach into the lower third of the abdomen. The free edge, however, is very active in adhesion formation as has been shown by Neuhof and Wiener.[11] In white rats the omentum is extensive but very delicate. Rubin, in his careful studies on the omentum,[12] states that this organ as well as its part in the formation of adhesions is very similar in cats, dogs, and men. The absence of adhesions in babies[2, 4] may be due partly to differences in age, since age influences the rate of absorption and the reaction to infection.[13] A comparison of pups and adult dogs would be interesting.

Our next attempt was to diminish the formation of adhesions. Among the many methods advocated by various surgeons,[9, 10] the method thought to be the most applicable to the present problem was the postural separation of surfaces likely to adhere. Because of the frequent involvement of the omentum and its lack of spontaneous motility,[12] it was thought that keeping the injected solution away from the usual location of this organ might reduce

the incidence of adhesion formation. For this purpose, dogs were kept on their backs in a "Fowler's position" (20° incline) for twelve hours after each injection into the lower left quadrant.

Adhesions were found in 4 of the 6 dogs injected. These adhesions, however, were not as marked as those obtained when the dogs were unrestrained. These were the only animals in the entire series showing any distress after injection. Their vomiting, however, was due to the injection being given in the evening when their stomachs were full, and was easily corrected. In three dogs the omentum was stretched clear to the symphysis and held by firm adhesions. This unusual condition is probably explained by the posture and by the use of morphine (for quieting), tending to favor a displacement of the viscera toward the symphysis.

Although keeping the solution away from the common site of the omentum materially reduced the incidence and degree of formation of adhesions, this method has little clinical value since low insertions of the omentum frequently cause chronic distress by pulling on the transverse colon and stomach.[14]

We next attempted to diminish adhesion formation by varying the solution injected. Variations in the tonicity of the solution and in the concentration of neoarsphenamine were tried because of their influence on the rate of absorption, on the exudation of body colloids, and possibly, on the direct damage to the mesothelial cells.[6] The hydrogen-ion concentration was varied because of its effect on the rate of oxidation and the coincident toxicity of arsphenamines.[8] Several solutions were used as vehicles because they might either reduce the irritant properties of neoarsphenamine or interfere with the formation of adhesions. As many experiments of each type as could be included in a single series were run to get an indication as to which theory should be tested more fully. When the striking results from the use of sodium bicarbonate were noted, all other experiments were stopped to run a series of such animals. As the problem had to be dropped shortly after this, the incomplete results from the other solutions are valuable chiefly as controls.

All solutions were prepared within an hour of the injection time. Sodium chloride solutions were boiled twenty minutes and made up to volume. Glucose solutions were autoclaved. Weighed portions of supposedly sterile sodium bicarbonate were folded in papers, inserted in a water-tight finger of an old rubber glove, and autoclaved; all cultures were negative. This last procedure was used because some of the bicarbonate heated in solution changes to the more irritating carbonate.[15] The neoarsphenamine was added to the solutions of the vehicle just before injection. This is important, in that, if the sodium bicarbonate increases the rate of oxidation of the neoarsphenamine when it is exposed to air,[16, 17] there would have been very little time, in our experiments, for such an action to have occurred. Likewise, there could have been but little development of acidity in the glucose solutions.[18] All vehicles were used in strengths corresponding to one-half, one, and two times isotonicity.

Variations in the tonicity and in the concentration of neoarsphenamine (0.1 to 1 per cent), produced no appreciable effect on adhesion formation.

In the glucose series, adhesions were formed in 5 of the 6 dogs and were about the same as those formed when only water was used. One of these, Dog 41, whose death was mainly due to pneumonia, had a small 2 mm. per-

TABLE—SUMMARY

ANIMAL		VEHICLE	PERITONEAL PATHOLOGY						
			ADHESIONS				PERITONITIS NO.	NO PATHOLOGY (PER CENT)	
			MARKED		SLIGHT				
SPECIES	NO.		NO.	(PER CENT)	NO.	(PER CENT)			
Guinea Pig	8	Water	0	0	0	0	0	100	Varying species
Rabbit	10	Water	0	0	0	0	0	100	
White Rat	6	Water	0	0	0	0	0	100	
Dog	7	Water	5	71	1	14	0	14	
Cat	7	Water	2	28	3	43	1	14	
Dog	7	Water	5	71	1	14	0	14	No restraint
Dog	6	Water	3	50	1	17	0	33	Dorsal; shoulders elevated 20° for 12 hours
Dog	7	Water	5	71	1	14	0	14	Varying vehicle
Dog	6	Glucose	4	67	1	17	1	17	
Dog	3	Ringers	1	33	0	0	2	0	
Dog	2	NaCl	0	0	1	50	0	50	
Dog	12	$NaHCO_3$	0	0	1	8	0	83	
Dog	4	½ Iso-	0	0	1	25	0	50	Varying tonicity and H-ion. All $NaHCO_3$
Dog	4	Iso-	0	0	0	0	0	100	
Dog	4	2 Iso-	0	0	0	0	0	100	

All received 0.012 gm. of neoarsphenamine per kilogram of body weight as a 1 per cent solution injected at intervals of four to seven days.

foration of the ilium leading to a cavity 4 by 1 mm., with a wall 1 mm. thick, backed by adjacent loops of the intestine and its mesentery.

Ringer's solution formed a milky precipitate when added to the neoarsphenamine, and 2 out of the 3 animals injected died from a severe chemical peritonitis. The use of this vehicle was not logical, but the result serves to emphasize the toxicity of a cloudy solution of neoarsphenamine.

The sodium chloride series suffered heavily in a pneumonia epidemic. One of the two animals surviving had a single firm adhesion after two injections.

In twelve dogs injected with neoarsphenamine in a sodium bicarbonate solution, only one delicate adhesion (between the omentum and the intestinal mesentery) was found. Such a low incidence is probably well within the normal range for the adult street dogs used. None of the animals showed any of the symptoms ascribed to the oxidized, toxic forms of neoarsphenamine.[8] One, Dog 76, died six days after the fifth injection from a gangrenous intussusception and peritonitis. This intussusception should be considered since among 19 dogs injected once, and so, not included in the present report, two, receiving water and one per cent sodium bicarbonate as vehicles respectively, died from the same cause. The first, however, had evidently had a piece of glass wedged firmly in its pyloris for some days. Moreover, it would seem that any irritation of the peritoneal surfaces by the neoarsphenamine would tend to decrease rather than increase peristalsis.[19] Possible etiologic factors of intussusception other than the intraperitoneal injection included: the mild stimulation of peristalsis during the excretion of arsphenamines;[8] intestinal worms, present in all three dogs and recognized as an etiologic factor;[20] then too, individual factors, since the peritoneal condition of these animals obtained from the dog pound could only be guessed at by their appearance. Injections made into the bowel wall of operated animals produced neither intussusception nor a perforation in any way similar to that found in Dog 41.

It is an open question why sodium bicarbonate should reduce adhesion formation. A physiologic action might be deduced from the very soothing effect similar solutions of sodium bicarbonate have on the nasal and pharyngeal mucous membranes. Changes in the physical state of the semicolloidal neoarsphenamine may have significance. A chemical reaction probably does not occur under the conditions used.

The therapeutic effect of neoarsphenamine may possibly be increased by injecting it intraperitoneally, in that, subcutaneous and intramuscular injections are similarly modified by body colloids, by the rate of absorption, and by their distribution, and are experimentally more parasiticidal than the intravenous.[21, 22] Moreover, if oxidation processes are increased by the alkalinity, then by Voegtlin's "arsenoxide theory,"[8, 23] we might expect some increase from this source. Then, too, possibly such combinations as occur with the peritoneal colloids would be beneficial by preventing the intravascular precipitation of organic arsenicals which Danysz[24, 25] considers the cause of the nitritoid reactions. That such combinations do not prevent absorption is shown by the clinical results quoted before. We have, moreover, some unpublished experimental results showing arsenic in the blood and lymph after intraperitoneal injection.

CONCLUSIONS

1. One per cent aqueous solutions of neoarsphenamine injected intraperitoneally cause adhesion formation in most dogs and cats, but not in guinea pigs, rabbits, or white rats.

2. The addition of 1 to 4 per cent sodium bicarbonate solution greatly reduces the incidence of adhesion formation following intraperitoneal injections of neoarsphenamine in dogs.

3. Conclusions which might be drawn from various accidents include: the fact that injections into the lower part of the abdomen which cause omental adhesions would probably lead to distress from traction on the transverse colon and stomach; that a cloudy solution of neoarsphenamine should not be injected intraperitoneally; that a patient with a possible paralytic ileus should not be injected intraperitoneally; that if adhesions are formed, obstruction may result; and that an accidental injection into the abdominal wall may cause death.

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REFERENCES

1. Rosenberg, Wm.: The Intraperitoneal Administration of Neoarsphenamine, a Preliminary Report, J. A. M. A. **82**: 682, 1924.
2. Sanford, H. N.: Treatment With Intraperitoneal Injection of Neoarsphenamine, Report of Ten Cases, J. A. M. A. **85**: 242, 1925.
3. Yampolsky, J., and Klugh, G. F.: The Treatment of Syphilis in Children, Description of Intraperitoneal Injection of Neoarsphenamine and Mercurosal, J. M. A. Georgia **15**: 1, 1926.
4. Grulee, C. G., Sanford, H. N., and Waldo, P. C.: Congenital Syphilis, Treatment by Intraperitoneal Injection of Neoarsphenamine, Report of Two Years Work, Am. J. Dis. Child. **35**: 47, 1928.
5. Hertzler, A. E.: The Genesis and Significance of Abdominal Adhesions, J. Tennessee M. A. **19**: 29, 1926.
6. Cunningham, R. S.: The Physiology of Serous Membranes, Physiol. Rev. **6**: 2, 1926.
7. Hertzler, A. E. The Peritoneum, St. Louis, 1919, The C. V. Mosby Co.
8. Voegtlin, C.: Pharmacology of Salvarsan and Related Arsenicals, Physiol. Rev. **5**: 63, 1925.
9. Adams, J. E.: Peritoneal Adhesions and Their Treatment, Practitioner **117**: 273, 1926.
10. Richardson, E. H. Studies on Peritoneal Adhesions, Ann. Surg. **54**: 758, 1911.
11. Neuhof, H., and Wiener, S.: Some Experiments on Omental Adhesions, Surg. Gynec. Obst. **10**: 348, 1910.
12. Rubin, I. C.: The Functions of the Great Omentum, Surg. Gynec. Obst. **12**: 117, 1911.
13. Pribram, B. O.: The Absorptive Capacity of the Peritoneum in Young and Old, Zentralbl. f. Chir. **51**: 1964, 1924.
14. Hibbitt, C. W.: Intra-abdominal and Intrapelvic Adhesions, Postoperative and Otherwise, Am. J. Surg. **39**: 1, 1925.
15. Epstein, J. W.: Intraperitoneal Administration of Sodium Bicarbonate Solutions (Preliminary Report), Arch. Pediat. **37**: 656, 1920.
16. Roth, G. B.: Effect of Shaking Alkalinized Aqueous Solutions of Arsphenamine and Aqueous Solutions of Neoarsphenamine in Presence of Air, Pub. Health Rep. **35**: 2205, 1920.
17. Schamberg, J. F., Kolmer, J. A., and Raziss, G. W.: Influence Upon Toxicity and Trypanocidal Activity of Shaking Acid and Alkalinized Solutions of Arsphenamine and Solutions of Neoarsphenamine in Air, Am. J. Syph. **6**: 1, 1922.
18. Stoddard, J. L.: Avoidance of Intravenous Glucose Reactions, Boston B. & S. J. **191**: 1121, 1924.
19. Macleod, J. J. R.: Physiology and Biochemistry in Modern Medicine, St. Louis, C. V. Mosby Co., ed. 5, p. 700.

20. Rose and Carless: Manual of Surgery, New York, Wm. Wood and Co., ed. 11, p. 1268.
21. Castelli, G.: Ztschr. f. Chemotherap. **1**: 122, 1913; **1**: 321, 1913.
22. Voegtlin, C., and Smith, H. W.: Quantitative Studies in Chemotherapy; Intravenous Versus Intramuscular Administration of Arsphenamine, Curative Power and Minimum Effective Dose, J. Pharmacol. & Exper. Therap. **17**: 357, 1921.
23. Voegtlin, C., Dyer, H. A., and Leonard, C. S.: On the Mechanism of the Action of Arsenic on the Protoplasm, Pub. Health Rep. **38**: 1882, 1922.
24. Oliver, J., Yamada, S. S., and Kolos, F.: Biologic Reactions of Arsphenamine; Reduction of Its Toxicity by Combination With Hydrophilic Colloids, Arch. Dermat. & Syph. **8**: 1, 1923.
25. Danysz, J.: Les Propriétés Physiochemiques des Produits du Groupe des Arsénobenzènes; Leurs Transformations dans l'Organisme, Ann. de l'Inst. Pasteur. **31**: 114, 1917; **31**: 483, 1917.

NOTE ON THE EFFECT OF LEAD ON RAT SARCOMA*

By Johannes S. Buck, Ph.D. and Donald M. Kumro, M.S., Ph.D.,
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DURING work on the toxicity of lead compounds, one of the rats developed a spindle-cell sarcoma. The point of interest is that the rat was heavily loaded with inorganic lead and developed the sarcoma in the presence of a marked plumbism, from which it ultimately died.

The following intraperitonal injections were given in the form of lead nitrate (calculated as metallic lead):

First injection (rat approximately three months old) 1.0 mg. per 100 gm. body weight.
Second injection (two months later) 1.5 mg. per 100 gm. body weight.
Third injection (five months after previous) 12 mg. per 100 gm. body weight.

The rat died seven months after the last injection and the autopsy showed two small tumors over the fascia, one the size of a pea and the other somewhat larger, apparently at the sites of injection.

The pathologic diagnosis on this tissue was made by Dr. W. M. Coppridge of Watts Hospital, Durham, N. C., and confirmed by Dr. A. A. Thibaudeau of the State Institute for the Study of Malignant Diseases, at Buffalo, N. Y.

Periodic examination of the blood of this animal showed marked polychromatophilia along with anisocytosis, poikylocytosis, achromia and including the appearance of normoblasts, indicating plumbism. Furthermore, some unabsorbed precipitated lead was found in the cavity.

Tissue examination of the liver showed dense adhesions to the diaphragm and a chronic inflammatory process between the lobules. The kidney showed a chronic diffuse nephritis, both glomerular and tubular, the destruction being severe.

The tumor may have been of spontaneous origin or may have been started by irritation due to the injection (other cases indicated the latter). In either case the development in the presence of excessive amounts of lead is noteworthy.

One case, naturally, proves nothing. It is intended to follow up the matter, but as it will take considerable time, this note is presented in the meantime.

*From the Department of Chemistry, Drake University.
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A NOTE ON THE SENSITIVITY OF THE KOLMER AND KAHN TESTS DURING CHILL AND FEVER*

By John H. Besancon, M.D., and Verna R. Mayer, Philadelphia, Pa.

IT HAS long been known that nonspecific Wassermann-positive reactions have been obtained in patients suffering from fevers. Korschun and Leibfreid[1] report 50 per cent positive reactions in recurrent fever; Eichelberg[2] reports 40 per cent positive reactions in scarlet fever cases; Weil and Braun[3] report positives in malaria. With the newer and more standardized and sensitive technics, however, nonspecific positive reactions do not occur so frequently as in the older technics and the specificity of the modern serologic tests for syphilis is being established. Kolmer[4] states that the sera in acute febrile diseases may become somewhat more anticomplementary than usual but with due care in technic these nonspecific positive Wassermann reactions can be avoided.

The treatment of a group of syphilitic patients with fever induced by nonspecific protein, offered an excellent opportunity to test the sensitivity of the Kolmer and Kahn tests during the chill and fever, and to compare these readings with those taken at normal temperature, to see if the blood shows a tendency to an increased or decreased sensitivity toward positivity in these morbid states.

The quantitative ice box modification of the Wassermann test described by Kolmer,[5] and the precipitation test described by Kahn,[6] were used and the principles outlined by each were adhered to.

A group of syphilitic patients at all stages of the disease, whose serologic tests varied from negative to strongly positive, were used. Fever was induced in these patients by the intravenous injection of typhoid, paratyphoid, streptococcus, colon and gonococcus vaccines. Blood was drawn in sterile dry syringes from the anticubital vein, immediately before the vaccine was intravenously injected, and again about one and a half hours later, when a chill occurred. These constitute the chill studies, which amount to 50 pairs of tests taken on 15 patients; i.e., a test at normal temperature and a test during chill in each instance. Likewise, for the fever series, blood was drawn at normal temperature, and again when the temperature reached 103° or above. This amounted to 50 pairs of tests taken on 18 patients. Each pair of specimens was tested at the same time in the same set-up, so that variations could not be attributed to technic.

Chill studies and fever studies were performed at different times, inasmuch as we had not decided to do the former until the latter study had been completed.

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RESULTS

Chill Group.—This group represents 50 Kolmer and Kahn tests taken at normal temperature and again during the chill. Thirty-four of these pairs, or 64 per cent, showed no change in the titer or intensity of the Kolmer Wassermann and Kahn tests between normal temperature and chill, whether the test at the outset was positive or negative. Sixteen, or 32 per cent, of the pairs showed variations in titer or intensity between that of normal temperature and that of chill in either Kolmer or Kahn test.

First Group.—The following table presents those cases in which the Kolmer remained unchanged while the Kahn varied:

	AT NORMAL TEMPERATURE		AT CHILL	
CASE	KOLMER	KAHN	KOLMER	KAHN
1	44400	000	44400	012
2	44400	344	44400	122
3	44400	444	44400	122
4	00000	122	00000	000
5	00000	122	00000	000

In Cases 1, 2, and 3 of this group, the Kolmer remains strongly positive; in 4 and 5 the Kolmer remains negative. In Case 1 the Kahn increases from negative to weakly positive. In Cases 2 and 3 the Kahn decreases from strongly positive to weakly positive. In Cases 4 and 5 the Kahn decreases from weakly positive to negative.

Second Group.—The following table presents those cases in which the Kahn remained unchanged while the Kolmer varied:

	AT NORMAL TEMPERATURE		AT CHILL	
CASE	KOLMER	KAHN	KOLMER	KAHN
1	21000	122	43100	122
2	01100	122	34400	122
3	22200	333	44400	333
4	01100	122	34400	122
5	12100	122	34400	122

In this group the Kahn remains weakly or moderately positive throughout, while the Kolmer increases from weakly positive to strongly positive.

Third Group.—The following table presents those cases where both the Kolmer and Kahn varied:

	AT NORMAL TEMPERATURE		AT CHILL	
CASE	KOLMER	KAHN	KOLMER	KAHN
1	33200	012	44400	333
2	23300	000	44400	122
3	11100	001	44000	344
4	44400	333	00000	000
5	44400	333	00000	000
6	44400	444	00000	000

The Kolmer and the Kahn tests vary in the same direction. In Case 1 the Kolmer increases from medium positive to strongly positive, while the

Kahn increases from weakly positive to medium positive. In Case 2 the Kolmer increases from medium positive to strongly positive, while the Kahn increases from negative to weakly positive. In Case 3 the Kolmer and Kahn both increase from weakly positive to strongly positive. In Cases 4, 5, and 6 the Kolmer and Kahn both decrease from strongly positive to negative.

Summary of Observations on Chill.—The above results show that five, or 10 per cent, of the Kolmer tests remained unchanged during the chill while the Kahn test varied. Adding this to the 64 per cent of unchanged Kolmer and Kahn tests makes a total of 74 per cent of the Kolmer tests which gave the same reading during chill as at normal temperature. Five, or 10 per cent, of the Kahn tests remained unchanged during chill, making a total of 74 per cent of the Kahn tests which were unaffected by chill.

Where there were variations, eight, or 16 per cent, of the Kolmer tests showed a tendency toward increased positivity during the chill; while three, or 6 per cent, showed a tendency toward negativity. In the Kahn variations, four, or 8 per cent, showed an increased tendency toward positivity, as compared with seven, or 14 per cent, which showed a tendency toward negativity.

We conclude from the above that chill definitely changes the sensitivity of the Kolmer and Kahn, since 26 per cent of both these tests showed variations in that state. The Kolmer reaction showed a tendency to greater positivity during the chill; while the Kahn reaction showed about the same tendency in the opposite direction toward negativity.

Fever Group.—This group represents 50 Kolmer and Kahn tests taken at normal temperature and again during the height of fever (103° or more). Twenty-seven of these pairs, or 54 per cent, showed no change in the titer or intensity of the Kolmer-Wassermann and Kahn tests between normal temperature and fever height, whether the test at the outset was weakly positive or strongly positive. Ten of these pairs, or 20 per cent, which were entirely negative at the outset, remained negative during fever height. This makes a total of 37 of these pairs, or 74 per cent, which gave the same reading throughout the experiment in both tests. Thirteen of the pairs, or 26 per cent, showed variation in titer or intensity between that of normal temperature and that of fever height in either Kolmer or Kahn test.

First Group.—The following table presents those cases in which the Kolmer remained unchanged while the Kahn varied.

In Cases 1, 2, 3, 4, 5, and 6 the Kolmer remains strongly positive. In Cases 7 and 8 the Kolmer remains negative. In Cases 1 and 2 the Kahn increases from negative to a weakly positive. In Case 3 the Kahn increases

	AT NORMAL TEMPERATURE		AT CHILL	
CASE	KOLMER	KAHN	KOLMER	KAHN
1	44000	000	44000	221
2	44430	000	44430	122
3	44200	233	44200	444
4	44100	443	44100	111
5	44400	444	44400	122
6	44400	444	44400	122
7	00000	333	00000	000
8	00000	333	00000	000

from a medium positive to strongly positive. In Cases 4, 5, and 6 the Kahn decreases from a strongly positive to weakly positive. In Cases 7 and 8 the Kahn decreases from strongly positive to negative.

Second Group.—The following table presents those cases in which the Kahn remained unchanged while the Kolmer varied.

	AT NORMAL TEMPERATURE		AT FEVER HEIGHT	
CASE	KOLMER	KAHN	KOLMER	KAHN
1	00000	122	00100	122
2	00000	000	21000	000
3	11000	122	44300	122
4	43100	122	00000	122

In Cases 1, 3, and 4 the Kahn remains weakly positive throughout. In Case 2 the Kahn remains negative. In Cases 1 and 2 the Kolmer increases from a negative to weakly positive. In Case 3 the Kolmer increases from a weakly positive to a strongly positive. In Case 4 the Kolmer decreases from a strongly positive to negative.

Third Group.—The following table presents the case in which both the Kolmer and Kahn varied:

	AT NORMAL TEMPERATURE		AT FEVER HEIGHT	
CASE	KOLMER	KAHN	KOLMER	KAHN
1	43000	333	00000	000

Both Kolmer and Kahn tests decrease from a moderately positive reaction to a negative one.

Summary of Observations on Fever.—The above results show that eight, or 16 per cent, of the Kolmers remained unchanged while the Kahn varied. Adding this to the 74 per cent of the tests where neither the Kolmer nor the Kahn varied during fever, makes a total of 90 per cent of the Kolmer tests which gave the same reading at fever height as at normal temperature. Four, or 8 per cent, of the Kahn tests remained unchanged during fever, making a total of 82 per cent of unchanged Kahns when added to the 74 per cent of Kahns which had remained unchanged in both tests during fever.

Three, or 6 per cent, of the Kolmers showed a tendency to increase toward positivity while two, or 4 per cent, showed a tendency toward negativity.

Three, or 6 per cent, of the Kahns increased toward positivity during fever height while six, or 12 per cent, showed a tendency toward negativity.

We conclude from the above that fever does not greatly affect the Kolmer test, there being only 10 per cent which showed variations; that the Kahn test is more greatly affected by fever, there being 18 per cent which showed variations. Where there were variations, the Kolmer test showed a slightly greater tendency toward positivity than negativity in fever. The Kahn test showed quite a definite tendency toward negativity in fever.

Comparing the behavior of the two tests in chill and fever, the Kolmer test showed a much greater stability in fever as compared to chill, there being 90 per cent unchanged Kolmers in the former as compared to 74 per cent in

the latter. The Kahns likewise showed a greater stability in fever as compared with chill, there being 82 per cent of unchanged tests in the former as compared with 74 per cent in the latter. The Kolmer test showed a much greater tendency toward positivity in chill, and very little tendency in fever; while the Kahn test showed a marked tendency toward negativity in both chill and fever.

SUMMARY

Two hundred Kolmer and Kahn tests were performed on a group of 33 syphilitic patients receiving fever therapy (nonspecific protein), whose serologic tests on the blood varied from negative to strongly positive reactions. The tests were performed on blood drawn at normal temperature, during the chill, and during the height of fever (103° or more). Serologic results in these morbid states were compared with those taken at normal temperature.

During the chill 74 per cent of both the Kolmer and Kahn tests gave the same readings as at normal temperature.

During the fever height, 90 per cent of the Kolmer tests gave the same readings as at normal temperature; while 82 per cent of the Kahn tests gave the same reading as at normal temperature.

In those series which showed variations the Kolmer test showed a tendency toward increased positivity during chill, there being 16 per cent which showed a tendency toward increased positivity as compared to 6 per cent which showed a tendency toward negativity; and very little tendency during fever height, there being 6 per cent which showed a tendency to increase toward positivity as compared with 4 per cent with a tendency toward negativity. The Kahn test showed a tendency toward negativity in both chill and fever, there being 8 per cent which showed a tendency toward positivity as compared with 14 per cent toward negativity in the former, while 6 per cent showed a tendency toward positivity as compared with 12 per cent toward negativity in the latter.

REFERENCES

1. Korschun, S., and Leifreid: Ueber Komplementbindung bei Typhus recurrens, Deutsche med. Wehnschr. **35**: 1179, 1909.
2. Eichelberg: Zur praktischen Verwertbarkeit der Wassermannschen Serumreaktion auf Lues und ueber das Vorkommen derselben bei Scharlach, Muenchen. med. Wehnschr. **55**: 1206, 1908.
3. Weil, E., and Braun, H.: Ueber die Entwicklung der Serodiagnostik bei Lues, Wien. klin. Wehnschr. **21**: 624, 650, 938, 1908.
4. Kolmer: The Specificity, Sensitiveness and Practical Value of the Kolmer-Wassermann Reaction, Am. J. Syph. **18**: 248, April, 1929.
5 and 6. Kolmer and Boerner: Laboratory Diagnostic Methods, New York, London, 1925, D. Appleton & Co.

TOXINS, ANTITOXINS AND DERMAGENS*

By L. G. Hadjopoulos, M.D., and Reginald Burbank, M.D., New York City

WHILE studying the relation of skin tests (diphtheria and scarlatina) to complement-fixation reactions, using the respective toxins as antigens, we came to observe certain facts that throw a new light on the nature of toxins as antigens and their serologic relationship to their respective antibodies.

With a very limited knowledge of the chemical nature of toxins, and still less of the antigenic radical in the complex protein structure, we still assume, in accordance with the generally accepted theory, that all proteins, and only proteins, contain the true antigenic (immunogenic) radical. If toxins were true antigens, they should contain a part if not all of the antigenic radical. It is certain that their parenteral introduction gives rise to specific antibodies, called antitoxins, which is also true with antigens in general. It remains, however, to be proved whether or not antitoxins can be considered true antibodies in all respects.

The Relationship of Skin Tests to Complement-Fixation Reaction.—Our study was limited to bacterial toxins in diphtheria, scarlatina, and certain streptococcic focal infections. The experimental data as given in Table I, though insufficient for drawing definite conclusions, are nevertheless indicative of the close relationship between the two apparently dissimilar serologic phenomena. In performing the skin tests, we have strictly adhered to the accepted standard methods, using the New York Department of Health products, except in the one instance of the streptococcic toxin which was produced according to the Banzhaf method of 65 per cent alcoholic saturation. The same toxins were used as antigens in the complement-fixation reaction. A hundred units of scarlatinal toxin, 0.01 of the L+ dose unit of diphtherial toxin and 0.01 mg. of focal streptococci were used per test. The active serum technic as performed by us was used throughout.

Comments on Table I.—Making due allowance for the limited number of experiments, it is nevertheless apparent that there is a close parallelism between the skin's sensitization and the complement-fixation tests. The only logical explanation for this phenomenon is the close relationship existing between the skin reagin and the antibody that freely circulates in the system in the above-mentioned pathologic states. Furthermore, toxin complement fixations are strictly specific in scarlet fever and diphtheria, and partly so in focal streptococcic infection because of the heterogeneous nature of the streptococcus species. Lastly, toxin complement fixations are little, if at all, affected by the presence of the Wassermann reagin in the same individual.

Now that a definite relationship has been established between the skin-sensitizing reagin and the complement-fixing reagin, it remains to be seen

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TABLE I

THE RELATIONSHIP BETWEEN SKIN TEST AND TOXIN COMPLEMENT-FIXATION TEST

	DEGREE TOXIN FIXATION IN COMPLEMENT UNITS	SKIN TESTS, DURATION IN DAYS	WASSERMANN REACTION
Scarlatinal Toxin			
1. M_3	++++	+++	−
2. K	+	±	−
3. S	++++	++	−
4. M_1	−	−	−
5. St_1	±	−	−
6. St_2	++++	+++	−
7. St_4	++	+	−
Diphtheria Toxin			
1.	++	+	++++
2.	+	+	−
3.	−	−	−
4.	−	−	+
5. Ch	++	++	−
6. GG	++	++	−
Focal Str. Toxin			
1. HK	+	+	−
2. PR	++	++	−
3. DB	+	+	−
4. C	++++	+++	−
5. AP	++	++	−
6. GK	+	+	−
7. CC_1	++++	+++	++++
8. CC_2	++++	+++	++++
9. DZ	++++	++	−
10. AG	+++	+++	−
11. LH	++++	+++	−
12. KK	−	−	−

NOTE: The plus system in the case of complement fixation denotes the degree of reaction in terms of fixed complement units; in the case of skin tests, the duration of reaction in days.

(1) whether this relationship is due to the two reagins being identical, or to the coexistence of both (antibodies) in the same patient, and (2) what relationship these reagins bear to antitoxins, i.e., the artificially produced antibodies against toxin inoculation.

TABLE II

IDENTITY OF THE SKIN AND COMPLEMENT-FIXING REAGINS

DATE	CLINICAL HISTORY	TOXIN COMPLEMENT FIXATION	SKIN TESTS
5/ 2/28	Date of onset of tonsillitis Hemolytic streptococcus isolated		
5/ 4/28	Toxin prepared	++++	+++
5/ 8/28	Cheesy foci in tonsils	++++	++++
5/11/28	Tonsillitis subsiding, cough	++	++
5/14/28	Afebrile, feeling normal	+	−
5/18/28	No symptoms	++++	++
5/22/28	Tonsillar relapse, moderate	++	−
6/15/28	Normal since May 25, no symptoms	++	−

Identity of the Skin and Complement-Fixing Reagins.—We have studied during the course of an acute tonsillitis the first aspect of the question. As antigen we used the autogenous toxin prepared from a strain of hemolytic streptococcus isolated from the tonsil of the patient. The results as recorded

chronologically in Table II strongly suggest that both reagins are identical, because the one increases or decreases simultaneously with the other. The complement-fixing reagin, however, seems to be the main source for both reactions as it is more lasting.

On account of this close similarity between the two reagins and owing to the lack of a proper nomenclature, we take the liberty of calling them collectively *dermagens.*

Is the Relation Between Toxin and Antitoxin That of Antigen and Antibody?—Antitoxins are reaction bodies produced artificially by the parenteral introduction of toxins. Dermagens are naturally found in the system of certain individuals predisposed to certain toxopathies, like diphtheria, tetanus, scarlatina, etc. Ontogenetically, antitoxins and dermagens are dissimilar bodies. It is peculiar, however, that the same antigen (the toxin) has two distinct antibodies: the antitoxin, directly produced either by experimental inoculation or naturally through disease, and the dermagen, preexisting apparently independently. How the dermagen originates in the system is beyond the scope of this work.

Thus the dermagens behave like true antibodies to their respective toxins. One might suppose that antitoxins are the true antibodies to the toxins as they are directly produced against toxin inoculation. Experimental data, however, do not substantiate this view. As will be seen in Table III, antitoxins cannot fix the complement in the presence of their respective toxins, but react antagonistically to dermagens. A mixture of toxin-dermagen-complement that would naturally result in the binding of complement is prevented from doing so on the addition of antitoxin, as will be seen in Table III.

TABLE III

THE ANTAGONISTIC EFFECT OF ANTITOXIN TO TOXIN COMPLEMENT FIXATIONS

CASES TESTED UNDER FOLLOWING PATHOLOGIC CONDITIONS	TOXIN-DERMAGEN COMP. FIXATION	TOXIN-DERMAGEN-ANTITOXIN COMP. FIXATION ANTITOXIN AMOUNTS C.C. PER TEST		ANTITOXIN ALONE WITH COMP. OF EACH SERUM	TOXIN-ANTITOXIN COMP. FIXATION
		0.02	0.04		
Scarlatinal Toxin					
1. M_3	++++	−	−		
2. K	+	−	−	−	
3. S	++++	++	−	−	
4. M_1	−	−	−	−	−
5. S_1	±	−	−	−	
6. S_2	++++	+	−	−	
7. S_4	++	±	−	−	
Diphtheria Toxin					
1. 1	++	+	−	−	
2. 2	+	±	−	−	
3. 3	−	−	−	−	−
13. 13	−	−	−	−	−
5. Ch	++	−	−	−	
6. GG	++	−	−	−	
Focal Streptococcic Toxin					
1. M_2S	++++	++	−	−	
2. ATS_2	+	−	−	−	
3. KK	−	−	−	−	−

The antagonistic effect of antitoxin on dermagen in the toxin-dermagen-antitoxin complex as illustrated in Table III admits of two possibilities: first, that antitoxin acts directly on the sister reagin, the dermagen (a view that could not be substantiated experimentally); second, that it acts directly on its progenitor, the toxin. If we accept the latter hypothesis we find ourselves in the difficult position of accepting toxins as true antigens. In the whole realm of true antigens there is not an instance where an antibody that is produced by the parenteral introduction of an antigen will antagonize the complement-binding property of the latter. This brings us to the last phase of our work, the investigation of the antigenic nature of toxins.

Are Toxins True Antigens?—Our present knowledge of the chemical structure of toxins is very rudimentary. They are present in the globulin fraction of bouillon culture media in which the respective toxogenic microorganism had grown for a number of days. Toxin (exotoxin) is not a part of the bacterial protoplasm but is a catabolic, excretory product. The bacterial protoplasm is a true antigen which on parenteral introduction gives rise to a specific complement-fixing antibody. The bacterial toxin does not give rise to a complement-fixing antibody, nevertheless it manifests certain important antigenic characteristics: its parenteral introduction gives rise to an antibody, the antitoxin, which acts antagonistically to the complement-fixing property of the toxin antigen. Although not true antigens, toxins exhibit an immunophilic antigenic radical capable of binding the complement in the presence of an independent reagin, the *dermagen*.

In a previous work[1] we have demonstrated the possibility of splitting true antigens into a protein (immunogenic) and a lipin (immunophilic) radical. The antigenic properties as exhibited by toxins surely are not immunogenic and if anything they are more immunophilic, while the unsplit bacterial protein that elaborates the toxin as a metabolic product exhibits both properties, as do all true antigens.

As further evidence in this respect we attempted to extract any lipin present in the purified and dried tonsillitic streptococcic toxin to see if such lipin extracts were immunophilic. The result of these tests we give in Table IV.

TABLE IV

THE COMPLEMENT-FIXING PROPERTY OF TOXIN LIPINS AND TOXIN RESIDUE

SERIAL NUMBER	WASSERMANN REACTION	WHOLE TOXIN FIXATION	TOXIN LIPIN FIXATION	TOXIN RESIDUE (DELIPINIZED) FIXATION
665	−	−	−	−
666	−	−	−	−
667	−	−	+	−
671	−	+	++	−
672	±	−	−	−
673	−	+	+	−
675	−	−	++	−
676	−	−	+	−
St_1	−	−	−	−
St_2	−	+++	++++	±
GC	−	++	++++	±
H	−	−	+	−
KK	−	+	++	−

From the above list we have excluded all Wassermann positive cases to eliminate the possibility of the nonspecific effect of the Wassermann reagin on lipogenic antigens in general. In the rest, our position is justified in assuming that the lipin fraction of toxins is the cause of complement deviation. The delipinized toxin was shown to be totally lacking in this respect. The comparison of the whole toxin fixations with the lipin and the delipinized residue fixations speaks in favor of this conclusion.

CONCLUSIONS

1. Toxins are partial antigens embodying only the immunophilic fraction. The bacterial protoplasm, on the other hand, is a true antigen with both immunogenic and immunophilic radicals.

2. The parenteral introduction of toxins produces certain reaction bodies, the antitoxins, which, though specific in nature, cannot be reckoned as true antibodies, since the interreaction between them and the toxins does not require complement.

3. The true antibody for toxins is a specific reagin freely circulating in the blood of certain toxin-susceptible individuals. The existence of such an antibody can be demonstrated either by means of the complement-fixation reaction or the particular skin tests. For lack of specific terminology we call this reagin the *dermagen* on account of its peculiar dermoactivity.

4. The significance of our last observation is that it provides, by means of the complement-fixation reaction, a uniform and more convenient method to the routine skin test in the determination of certain toxopathies.

REFERENCE

1. Hadjopoulos, L. G., and Burbank, R.: The Origin and Nature of the Wassermann Antigen; The Duality of the Antigenic Nature of Erythrocytes, J. LAB. & CLIN. Med. **12**: 973-982, July, 1927.

6 EAST SEVENTY-EIGHTH STREET.

A STUDY OF THE TOXICITY OF STRONTIUM AND COMPARISON WITH OTHER CATIONS EMPLOYED IN THERAPEUTICS*

By David Loeser and A. Lincoln Konwiser, New York

VULPIAN (1885) is credited with having introduced strontium as a remedy. J. V. Laborde (1890) proved that it was nontoxic and harmless when pure. Since then, strontium has been employed in medicine by oral administration in the place of sodium and potassium cations as a carrier for a number of anions such as bromide, iodide, salicylate, lactate and others. As with potassium salts, the anions are evidently the active therapeutic agents for the reason that both potassium and strontium are only slightly and irregularly absorbed from the digestive tract. This probably accounts for the failure to establish more convincing evidence in the literature as to the therapeutic value of strontium.

Strontium is a divalent in the same chemical group as the alkali earths magnesium, calcium and barium. A number of pharmacologists have studied the effect of strontium on cells, isolated tissues and blood and compared the effects with those exerted by other elements including members of the same group. The impression gained from a research of these reports is that it resembles more nearly the effect of calcium, but that it is weaker. Sollmann[1] states: "This cation resembles calcium and may take its place in some of its characteristic pharmacologic relations; but it is much weaker and also less toxic."

Like calcium, strontium hastens the coagulation of the blood. This is not true of barium or magnesium. It is eliminated by the intestines. The urine contains only traces of strontium when given subcutaneously. It is said to increase the elimination of uric acid and have an effect on bony tissues which are imperfectly calcified. Like calcium, it exerts a digitalis-like action on the heart, but weaker. Paralysis of the heart by quinine and arsenic is said to be neutralized by strontium and calcium.

There have appeared in recent literature, reports of the intravenous injection of strontium salts, particularly of the bromide. These reports indicate its application in the treatment of urticaria and other skin affections, parathyroid tetany, spasmophilia, etc., and other conditions indicating the relationship to the effect of calcium.

A review of the literature on strontium reveals a lack of information regarding its action and toxicity when administered intravenously. We have deemed it desirable that experiments be carried on to determine the definite toxicity of strontium and to compare, if possible, the toxicity and tolerance with the elements more commonly employed in therapeutics such as sodium, potassium, magnesium, and calcium. We submit herewith the tabulated results. We employed white rats and the same technic of intravenous injection we described in 1924.[2]

*From the Loeser Laboratory, New York.
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Table I represents the results of the intravenous injection of strontium bromide in the form of a five per cent solution indicating the doses, grams per kilo weight, equivalent dose for human of 70 kilos and the amount of elemental strontium and bromine grams per kilo weight.

TABLE I

TOXICITY TESTS: STRONTIUM BROMIDE 5 PER CENT—$SrBr_2.6H_2O$

RAT NO.	DOSE GRAM/K	EQUIV. HUMAN DOSE (70K)	WEIGHT OF RAT	C.C. INJECTED	STRONTIUM G/K	BROMINE G/K	COMMENT
1084	0.250 Gm.	17.5 Gm.	150 Gm.	0.75 c.c.	0.061 Gm.	0.112 Gm.	
1085	0.375	26.25	132	1.00	0.092	0.169	
1100	0.450	31.50	135	1.22	0.110	0.202	
1086	0.500	35.00	260	2.60	0.123	0.225	
1096	0.500	35.00	140	1.40	0.123	0.225	Died 45 min.
1099	0.500	35.00	115	1.15	0.123	0.225	
1097	0.550	38.50	130	1.43	0.135	0.247	Died 2 min.
1098	0.615	43.05	120	1.48	0.151	0.276	Died on table
1087	0.750	52.5	200	3.00	0.185	0.337	Died on table

With the smaller doses, the animals reacted with the same symptoms following the intravenous injection of sodium bromide, becoming stupefied and remaining lethargic for a long time after recovery from the shock of the injection. It will be observed, however, that the toxicity of strontium bromide is greater than that of sodium bromide. White rats tolerate up to 2.50 gm. per kilo of sodium bromide, whereas our maximum tolerated dose is barely 0.5 gm. per kilo of the strontium salt. With the larger doses, the rats struggled violently toward the end of the injection, the hind leg becoming paralyzed before death ensued.

In view of the evident sedative effect of the bromide anion, we believed it desirable to ascertain the toxicity of strontium chloride and strontium iodide and arrive, if possible, at results which would clearly indicate the toxicity of strontium cation with due consideration for the effect of the accompanying anions.

Table II represents the results of the intravenous injection of strontium chloride in the form of a 5 per cent solution.

TABLE II

TOXICITY TESTS: STRONTIUM CHLORIDE 5 PER CENT—$SrCl_2.6H_2O$

RAT NO.	DOSE GRAM/K	EQUIV. HUMAN DOSE (70K)	WEIGHT OF RAT	C.C. INJECTED	STRONTIUM G/K	CHLORINE G/K	COMMENT
1088	0.250 Gm.	17.5 Gm.	123 Gm.	0.60 c.c.	0.082 Gm.	0.066 Gm.	
1089	0.300	21.0	150	0.90	0.098	0.079	
1104	0.300	21.0	135	0.81	0.098	0.079	
1101	0.350	24.5	140	0.98	0.115	0.093	
1103	0.375	26.25	200	1.50	0.123	0.100	Collapsed but recovered
1102	0.425	29.75	145	1.24	0.139	0.113	Died in 2 min.
1090	0.500	35.00	205	2.05	0.164	0.132	Died in 15 min.
1091	0.625	43.75	138	1.73	0.205	0.165	Died in 3 min.

These rats exhibited much more toxic symptoms. Their struggles were much more violent; great difficulty in breathing and violent twisting of tail before final effect of lethal dose. Greatest tolerance is approximately 0.350 gm. per kilo.

Table III represents the results of the intravenous injection of strontium iodide in the form of a 5 per cent solution.

TABLE III

TOXICITY TESTS: STRONTIUM IODIDE 5 PER CENT—$SrI_2.6H_2O$

RAT NO.	DOSE GRAM/K	EQUIV. HUMAN DOSE (70K)	WEIGHT OF RAT	C.C. INJECTED	STRONTIUM G/K	IODINE G/K	COMMENT
1092	0.250 gm.	17.5 gm.	125 gm.	0.62 c.c.	0.049 gm.	0.141 gm.	No visible effect
1093	0.375	26.25	130	0.97	0.074	0.211	
1105	0.425	29.75	110	0.94	0.084	0.240	
1094	0.500	35.00	160	1.60	0.098	0.282	Died 60 hr.
1106	0.500	35.00	135	1.35	0.098	0.282	
1108	0.550	38.50	130	1.43	0.108	0.310	
1107	0.625	43.75	110	1.38	0.123	0.352	Died 10 min.
1095	0.625	43.75	110	1.38	0.123	0.352	Very toxic

The rats did not struggle during the injection, and as a whole the solution appeared to be least immediately toxic. Rats which survived regained their previous weight within one week and retained their weight for another week while under observation. The minimum lethal dose of strontium iodide is apparently 0.625 gm. per kilo.

Table IV presents for comparison the approximate minimum lethal dose of strontium bromide, strontium chloride and strontium iodide, the equivalent strontium per kilo and the equivalent haloids per kilo, their atomic weight and the ratio of atomic weights to lethal dose.

TABLE IV

COMPARISON OF MINIMUM LETHAL DOSE

	$SrBr_2.6H_2O$	$SrCl_2.6H_2O$	$SrI_2.6H_2O$
Dose per kilo	0.5 Gm.	0.375 Gm.	0.625 Gm.
Strontium per kilo	0.123	0.123	0.123
Haloid per kilo	0.225	0.100	0.352
Atomic weight of halogen in even figures	80	35.5	127
Ratio of atomic weights halogens	2.25	1	3.55
Ratio of lethal dose haloid	2.25	1	3.52

A comparison of the approximate minimum lethal dose established very definitely that the toxicity of strontium bromide, chloride and iodide is essentially dependent upon the strontium content. In all three instances, the strontium per kilo weight is 0.123 gm. This is further confirmed by the fact that the haloid constituents at the minimum lethal dose are in the ratio of their atomic weights.

Table V indicates the minimum lethal dose of sodium bromide, chloride and iodide.

TABLE V

MINIMUM LETHAL DOSES OF SODIUM SALTS (10 PER CENT SOLUTIONS USED)

	NaBr	NaCl	NaI
Dose per kilo	2.5 gm.	1.8 gm.	1.3 gm.
Dose haloid per kilo	1.94 gm.	1.08 gm.	1.10 gm.

Comparing the minimum lethal dose of the respective salts of sodium and strontium, we find:

On a $6H_2O$ basis:

Strontium Bromide is 5 times more toxic than sodium bromide.
Strontium Chloride is 4.8 times more toxic than sodium chloride.
Strontium Iodide is 2.08 times more toxic than sodium iodide.

On an anhydrous basis:

Strontium Bromide is 7.2 times more toxic than sodium bromide.
Strontium Chloride is 8.1 times more toxic than sodium chloride.
Strontium Iodide is 3.0 times more toxic than sodium iodide.

In a previous paper,[2] we drew attention to the relative toxicity of potassium as compared to sodium as deciding our choice of sodium iodide for the intravenous administration of massive doses of iodides. In this instance, we employed a 10 per cent weight to volume solution as the solution employed clinically was of that concentration. For a comparison of the toxicity of strontium to that of potassium, we chose the chlorides and continued the choice of the chloride for comparison with the elements in the same group. We maintained a concentration of 5 per cent except in the case of magnesium and of barium which exception will be noted later.

We submit Tables VI, VII, VIII, and IX as indicating the toxicity of potassium, magnesium, calcium, and barium.

TABLE VI

TOXICITY TESTS: POTASSIUM CHLORIDE 5 PER CENT—KCl (0.67 N)

NO. RATS INJECTED	C.C. PER 100 GM.	GM. KCl PER KILO.	POTASSIUM G/K	CHLORINE G/K	COMMENT
4	0.120	0.060	0.031	0.029	Fairly well tolerated
1	0.150	0.075	0.039	0.036	Very sick
3	0.200	0.100	0.052	0.048	All died within 5 minutes

The minimum lethal dose is approximately 0.090 gm. KCl per kilo.

Lower doses produced symptoms of extreme agitation or uneasiness in rats after release from board. Toxic doses produced convulsive contortions, then coma, noted slight twitching of paws, heartbeat irregular for three to five minutes before death ensued.

TABLE VII

TOXICITY TESTS: MAGNESIUM CHLORIDE ($MgCl_2.6H_2O$ 2.06 PER CENT) (0.2025 N)

RAT NO.	C.C. INJECTED PER 100 GM.	GM. $MgCl_2.6H_2O$ KILO	MAGNESIUM G/K	CHLORINE G/K	COMMENT
1110	0.80	0.164	0.019	0.058	Survived
1122	0.80	0.164	0.019	0.058	Survived
1109	0.85	0.176	0.021	0.061	Died within 5 min.
1111	0.85	0.176	0.021	0.061	Died within 5 min.
1120	0.85	0.176	0.021	0.061	Died within 4 min.
1121	0.85	0.176	0.021	0.061	Died within 3 min.
1123	0.85	0.176	0.021	0.061	Died within 3 min.

This clearly indicated that $MgCl_2.6H_2O$ is lethal to white rats in doses of 0.176 gm. per kilo. In all fatal cases, the animals passed through the following stages: violent convulsions, twisting of tail, coma, no visible breathing, irregular heartbeat which continued for some time after respiration ceased.

TABLE VIII

TOXICITY TESTS: CALCIUM CHLORIDE—5 PER CENT—$CaCl_2$ (0.901 N)

NO. RATS INJECTED	C.C. PER 100 GM.	GM. $CaCl_2$ PER KILO	CALCIUM G/K	CHLORINE G/K	COMMENT
2	0.142	0.071	0.025	0.046	No immediate reaction
4	0.250	0.125	0.045	0.080	Increased respiration. Tendency to minor convulsions
5	0.284	0.142	0.051	0.091	Increased respiration. Convulsions and toxic symptoms
6	0.322	0.161	0.058	0.103	Very toxic—three immediate deaths
5	0.356	0.178	0.064	0.114	Three immediate deaths. Two barely survived

The minimum lethal dose is approximately 0.170 gm. $CaCl_2$ per kilo.

At lower doses noted increased respiration. Toxic doses produced convulsive contortions, gasping for breath and then coma for short period.

It will be observed from Table IX that when employing a 5 per cent solution of barium chloride, death ensues rapidly after injecting as small a volume as 0.05 c.c. per 100 gm., making it practically impossible to secure accurate measurement. We were of necessity compelled to employ a dilution of 0.5 per cent. The results are indicated in Table X.

TABLE IX

TOXICITY TESTS: BARIUM CHLORIDE—5 PER CENT—$BaCl_2$ (0.48 N)

NO. RATS INJECTED	C.C. PER 100 GM.	GM. $BaCl_2$ PER KILO	BARIUM G/K	CHLORINE G/K	COMMENT
1	0.20	0.100	0.066	0.034	Died immediately
1	0.15	0.075	0.049	0.026	Died immediately
1	0.10	0.050	0.033	0.017	Died immediately
1	0.05	0.025	0.016	0.009	Died within 30 sec.

TABLE X

TOXICITY TESTS: BARIUM CHLORIDE—0.5 PER CENT $BaCl_2$ (0.048 N)

NO. RATS INJECTED	C.C. PER 100 GM.	GM. $BaCl_2$ PER KILO	BARIUM G/K	CHLORINE G/K	COMMENT
1	0.40	0.020	0.013	0.007	Died immediately
1	0.30	0.015	0.010	0.005	Survived
1	0.20	0.010	0.007	0.003	Survived

The minimum lethal dose is about 0.020 Gm. $BaCl_2$ per kilo. Since deaths occurred quickly it was difficult to ascertain immediate cause. The dying animals ran the gamut of violent contortions and twisting of tail, and then ceasing of respiration and heartbeat. For a period of one minute after death, there persisted a twitching or tremor of injected leg or lower jaw, other signs of life having disappeared.

We had at the outset determined to confine our comparisons with the cations employed in therapeutics. We were prompted to extend our studies to barium which is rarely used in therapeutics, for the reason that the toxicity of magnesium, calcium, and strontium was in an order inversely to the increase

in atomic weights. Barium is generally recognized as the most toxic of the elements of this group and we believe it to be of interest to include it in these comparisons. It will be observed that the lowering of toxicity with the increase of atomic weight does not hold as to barium.

We submit herewith a summary of the toxicity of the chlorides of sodium, strontium, calcium, potassium, magnesium, and barium, placing them in the order of their increasing toxicity. It will be observed that at the concentration we employed, strontium occupies a position next to sodium, being less toxic than the other cations employed in therapeutics.

SUMMARY OF TOXICITY TESTS OF CHLORIDES

	APPROX. MINIMUM LETHAL DOSE PER KILO ANHYDROUS SALT	CATION	ANION (Cl)
Sodium Chloride (NaCl)	1.08	0.425	0.655
Strontium Chloride ($SrCl_2$)	0.223	0.123	0.100
Calcium Chloride ($CaCl_2$)	0.170	0.061	0.109
Potassium Chloride (KCl)	0.090	0.046	0.044
Magnesium Chloride ($MgCl_2$)	0.082	0.021	0.061
Barium Chloride ($BaCl_2$)	0.02	0.013	0.007

THE ANTAGONISTIC EFFECT OF STRONTIUM TO MAGNESIUM

In view of the affirmed similarity of strontium action to that of calcium and of the interest recently displayed in the antagonistic effect of calcium on the respiratory depressive action of magnesium, we carried on the following experiments. In order to avoid complications by the evident sedative effect of the bromide anion, we selected magnesium chloride and strontium chloride for this purpose.

In Table VII, we demonstrated that magnesium chloride ($6H_2O$) is lethal in doses of 0.176 gm. per kilo. In order to test out the effect of strontium, we prepared a similar solution containing in addition strontium chloride on a 1/5 equivalent basis.

Table XI indicates the results of the injection of a solution containing magnesium and strontium.

TABLE XI

TOXICITY TESTS: 2.06 PER CENT MAGNESIUM CHLORIDE ($MgCl_2.6H_2O$.2025 N) 0.55 PER CENT STRONTIUM CHLORIDE ($SrCl_2.6H_2O$.0405 N)

RAT NO.	C.C. INJECTED	WEIGHT OF RAT	GM. $MgCl_2.6H_2O$ KILO	GM. $SrCl_2.6H_2O$ KILO	COMMENT
1112	1.14	133	0.176	0.047	Survived
1113	1.03	120	0.176	0.047	"
1124	.98	115	0.176	0.047	"
1125	1.28	150	0.176	0.047	"
1126	.98	115	0.176	0.047	"
1114	1.05	110	0.195	0.052	Died 3 min. symptoms of magnesium alone

While these rats struggled toward the end of the injection, they recovered quickly from the shock and did not go into coma.

It will be observed in Table VII that all five rats died when injected with 0.176 gm. of magnesium chloride per kilo. When 1/5 equivalent of

strontium chloride was injected with the same dose of magnesium chloride, all the rats survived whereas the same equivalent of strontium chloride was incapable of antagonizing the effect of 0.195 magnesium chloride.

Meltzer and Auer[3] failed to demonstrate that strontium neutralizes the inhibitory effect of magnesium. These authors, however, employed the subcutaneous method of injection into the rabbit, an animal evidently less sensitive to magnesium. They state, however, that strontium causes a slight improvement of the respiration, but seems rather to aggravate and hasten other inhibitory symptoms due to magnesium, especially the paralysis.

To ascertain the effect of a smaller quantity of strontium, we prepared a solution containing 1/10 of equivalent strontium; 2.06 per cent magnesium chloride (0.2025 N), 0.275 per cent strontium chloride (0.02025 N).

At a dose of 0.176 gm. magnesium chloride ($MgCl_2$) one rat died within three minutes after going into coma. Another rat struggled violently, passed into coma with irregular breathing and heartbeat, finally recovering. It was evident that it requires a larger amount of strontium to neutralize the effect of the lethal dose of magnesium. We have shown, however, that strontium like calcium, in relatively small quantities, does exhibit an antagonistic effect to magnesium.

SUMMARY

We have ascertained by intravenous injection into animals that the minimum lethal dose of strontium is approximately 123 milligrams per kilo when the chloride, bromide, and iodide salts are employed.

We have compared the toxicity of strontium with that of sodium, the least toxic of the cations employed in therapeutics. By continuing the comparison with potassium and elements in its own periodic group, we have established that strontium is among the least toxic of the therapeutic cations, occupying the second position in the following order of increasing toxicities: sodium, strontium, calcium, potassium, magnesium, and barium.

In view of the extended clinical administration of strontium bromide and the comparatively low toxicity of strontium demonstrated by these experiments, it is to be concluded that strontium is adaptable for intravenous administration, providing properly controlled solutions and doses are employed.

We have demonstrated also that strontium antagonizes the toxic effect of magnesium by counteracting the respiratory depressive action thus corroborating the impression that the action of strontium resembles that of calcium.

REFERENCES

1. Sollmann: Manual of Pharmacology, ed. 3, 1926, W. B. Saunders Co., Philadelphia.
2. Loeser, David, and Konwiser, A. L.: Preliminary Note on the Difference in Pharmacologic Action of Potassium and of Sodium Salts When Administered Intravenously, J. Lab. & Clin. Med. 9: 385, March, 1924.
3. Meltzer and Auer: J. Physiol., p. 449, 1908.

A NOTE ON BLOOD CHEMISTRY*

By R. S. Hunt, Ph.D., Boston, Mass.

IN MAY of the current year I completed the chemical analysis of 262 samples of blood taken from members of the last five freshman classes of the Boston University School of Medicine. This work was done as part of the Vital Function Studies described by Rowe[1] and carried out yearly since its inauguration.

Since the subjects from whom the bloods were taken are on the whole, normal young men and women (chiefly men) of an average age of twenty-three years, living the life of the average college student the figures obtained may be of interest and are given in Table I. All bloods were taken after a fasting period of at least twelve hours' duration.

TABLE I

AVERAGED VALUES EXPRESSED IN MG. PER 100 C.C. OF WHOLE BLOOD†

YEAR	NPN	UREA N	URIC ACID	CREATININE	SUGAR
1925	31	16	3.7	1.4	98
1926	33	16	3.6	1.4	96
1927	32	16	3.3	1.3	99
1928	33	16	3.7	1.4	97
1929	31	15	3.8	1.5	98
Average (5 yr.)	32	16	3.6	1.4	98

†Note. The Folin-Wu system of blood analysis was used throughout with the exception of the uric acid determination which was that of Benedict-Franke.

Table II shows the percentage falling within normal limits as defined therein.

TABLE II

NPN	UREA N	URIC ACID	CREATININE	SUGAR
25 – 35 mg.	12 – 20 mg.	2.5 – 4.0 mg.	0.5 – 1.5 mg.	80 – 120 mg.
92.4%	95.8%	82.9%	95.8%	99.6%

TABLE III

	NPN	UREA N	URIC ACID	CREATININE	SUGAR
High	42	28	5.2	2.0	134
Low	24	10	2.4	1.1	81

No explanation is offered for the high and low values obtained. The individuals are still in the medical school or are interns in the Hospital and have shown no organic impairment at any time or have other function tests as applied given any evidence of renal impairment.

REFERENCE

1. Rowe, A. W., et al.: Vital Function Studies. II A Group Study of Certain Blood and Urine Findings, and Gaseous Metabolism, Boston M. & S. J. **192**: 747-752, 1925.

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STUDIES IN THE ALIMENTARY TRACT OF MAN*

III. The Gastric Responses to Milk and Buttermilk

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INTRODUCTION

IN PREVIOUS essays we[1, 2] have dwelt upon the necessity of reducing to a minimum the influence of central stimuli in the gastric reaction pattern before this is employed in experiments upon the human subject. We have shown how the effect of this influence can be quantitatively measured and how by an adequate course of training its disturbance of the gastric response can be rendered negligible. In the present article we shall set forth the results of our experiments on feeding diagnostic meals to stabilized stomachs.

GASTRIC MOTILITY

It is our intention to refrain from entering into discussion of the theory of tone. In our experience tone and peristalsis are both expressions of gastric motility. We acknowledge the clinical pictures of orthotony, hypertony and hypotony but we emphatically deny that these terms are adequate to define gastric tone. For us they imply nothing whatever regarding the state of tone: they are merely conventional phrases indicating differences in the length-phase of gastric musculature. Peristalsis is not necessarily less vigorous in hypotony nor more active in hypertony. Both tone and peristalsis are affected by obscure influences which, for want of a better term, we call psychological. Experiments discussed in our previous articles have separated the effect of this psychological influence from the direct reflex response of the stomach itself.

PERISTALSIS

Immediately upon swallowing the barium meal, our stabilized and trained stomachs show one or more zones of irritability (tension rings) on both curvatures or on the greater curvature alone. After some seconds these zones begin to pulsate and shortly thereafter propagate waves which pass toward the pylorus in orderly sequence and at a rate which shows little, if any, variation from one individual to another. In the final phase the pulsating zones disappear as such, and peristaltic waves alone are to be seen. When pulsation appears in the indentations it tends to occur in all at the same time, but peristalsis commences at the zone nearest the pylorus and with each successive wave, originates progressively in the zone nearer the cardia, until the pulsation

*From the Anatomical Laboratory Western Reserve University, Cleveland, Ohio.

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nearest the Magenblase is propagating its wave. We have never seen a preliminary inhibitory phase in advance of the peristaltic wave.

The usual positions of pulsating zones are at the junctions of the three main stomach divisions, gastric tube, pyloric vestibule and pyloric canal, and in the mid-gastric tube. Less frequently we see a pulsating zone in the upper gastric tube just below the Magenblase though this may be due to difficulty of discerning a zone in this relatively faintly outlined area. The size of meal seems to bear a relation to the number of pulsating zones. With a 16-ounce meal we have rarely seen more than one zone, and that at the junction of pyloric canal and vestibule or perhaps in the mid-vestibule. But with a 5-ounce meal we have frequently noted a pulsating zone as high as mid-tube. This observation is probably to be correlated with Trendelenburg's finding that peristaltic activity is stimulated by distension under pressure but that although peristalsis tends to be increased by pressure it rapidly becomes inhibited by over-distension.

Timing the peristaltic waves by stop watches we find speed from particular gastric landmarks remains constant with almost no individual variation. One wave may pulsate longer in the zone of its origin but the time required for its transmission to the pylorus does not change. A wave beginning at the junction of pyloric vestibule and canal takes 7 seconds to reach the pylorus; one beginning in mid-vestibule takes 15 to 18 seconds; if the wave starts at junction of tube and vestibule it takes 32 to 35 seconds to reach the pylorus. With a regular milk meal at 70° F. we very seldom see more than two waves at once in the stomach and the time interval between these is about 20 seconds so that the first is already passing through the pyloric canal as the second encroaches upon the pyloric vestibule. It is not uncommon to see a peristaltic wave which has started in the gastric tube die out on the vestibule and reappear in the pyloric canal, the time interval remaining unchanged. Increase in speed of peristalsis seems to be an illusion produced by the presence of successive waves: it is the time interval between them which is reduced. There is great variation in amplitude of the contraction in different subjects, experiments or conditions. It is diminished, for example, by physical weariness, increased by buttermilk. It varies in amplitude even in the successive divisions of the stomach. Sometimes amplitude is greatest at the origin of the wave in the gastric tube but usually increases as the wave approaches the pylorus.

There is a manifestation of irritability, somewhat resembling an ill-coordinated peristalsis, in which waves of minimal amplitude occur: we have called it shimmer. It usually occurs on the greater curvature alone, though more rarely it is seen equally on both curvatures. No definite orderly progress can be identified in these wavelets but the entire outline of the greater curvature seems to flicker and the shallow wavelets appear in such rapid succession as to give the impression of stationary pulsating minute indentations. In a very active buttermilk stomach where waves are of considerable amplitude it is not uncommon to find shimmer superposed in each large peristaltic wave.

When peristalsis is visible on the lesser curvature it is best seen in the pyloric canal and is then obviously a part of the wave of contraction passing along the greater curvature. When the lesser curvature wave is independent

of that on the greater it seems to have a rhythm of its own. Its amplitude is always less than on the greater curvature. When peristalsis is deep in the pyloric canal it is evident on both curvatures as a circular zone of contraction. We seldom see movement on the lesser curvature proximal to the incisura and, when it is seen higher up it often occurs as a shimmer immediately above the incisura angularis. The lesser curvature cannot be seen so distinctly as the greater and this may partially account for the scantiness of our information on lesser curvature peristalsis.

Gastric peristalsis invariably ceases at the pylorus. We find no evidence whatever for the view that the wave in the pyloric canal is distinct from that in the rest of the stomach.

In presenting the foregoing summary which is intended to describe the full series of successive phenomena observed in a trained and stabilized stomach, we must make the reservation that these phenomena do not invariably appear in complete expression. They may indeed sometimes pass through successive phases so quickly as to be unrecognizable or apparent only as a foreshortened sequence with definite phases omitted. Further the description cannot be forced to fit the erratic and often bizarre appearances which are to be found in normal but untrained or non-stabilized stomachs.

DIAGNOSTIC MEALS

With stabilized trained stomachs as our working material we set out to investigate the effect of barium vehicles, milk and buttermilk, on the stomach. On the fluoroscopic screen one has no difficulty in seeing the marked contrast between the effects of milk and buttermilk on the stomach. But as the fluoroscopic appearance does not lend itself to permanent pictorial record, observations on area of stomach shadow must be made by radiograms which, in their turn, give merely chance evidence of motility.

The subjects are given a "5-ounce meal," consisting of four ounces by volume of milk or buttermilk, at a temperature of seventy degrees Fahrenheit, with thirty-three grams of barium sulphate. The conditions of examination are constant so that radiograms are exactly comparable.

On the fluoroscopic screen buttermilk is seen to induce, immediately, gastric elongation and lateral distension with increased amplitude of peristalsis and passage. Sometimes gas in the flexures of the large bowel or in the transverse colon causes indentations which hold up contents temporarily in the cardiac sac. The stomach may be prevented from elongating directly downward. If so, the elongation takes place obliquely so that the greater curvature is swung over to the right of the vertebral column, thus changing the whole shadow outline and giving the illusion of a shorter stomach. In oblique elongation passage is not so clearly seen for the second part of the duodenum is hidden by the distal pyloric vestibule and canal but contents can readily be seen in the jejunum. Typical buttermilk waves are wide and deep, and pass along the entire contents with a regular rhythm.

Milk, on the contrary, usually enters the stomach more slowly than buttermilk. It gradually progresses to the pylorus with relatively little lateral distension or elongation. As soon as the milk contents reach the pylorus a rapid

shimmering movement is set up along the greater curvature in the pyloric vestibule. In some stomachs this is the sole evidence of activity: in others the shimmer gradually becomes deeper until it is transformed into shallow waves. The first wave usually appears in the pyloric canal but succeeding ones start in the progressively proximal sites until in due course they travel the entire length of the stomach. The gradually sinking level of the barium contents during the first ten minutes after ingestion indicates that passage is actually occurring although during this period it can be seen in the milk stomach only by a trained eye to which it is evident as a faint puff.

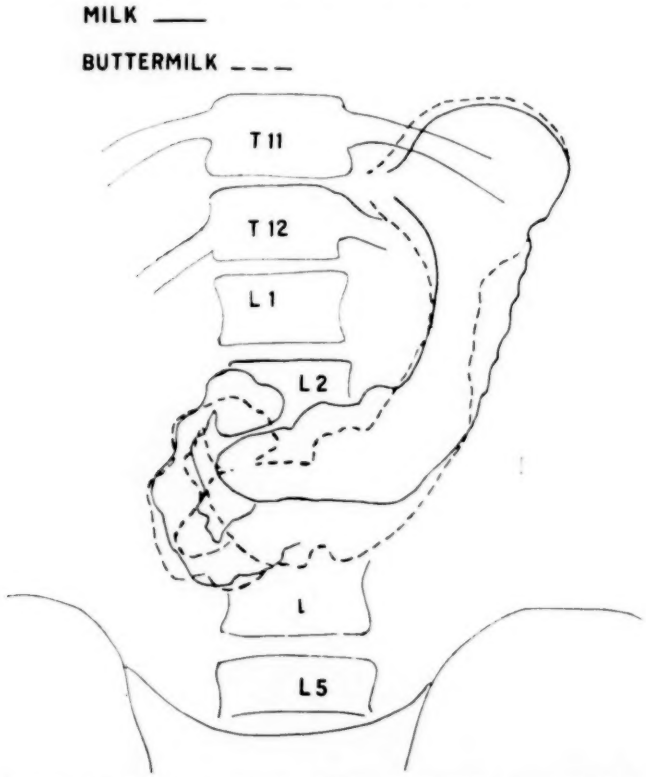

Fig. 1.—Barium vehicles and stomach outline. Buttermilk is associated with a larger, longer, usually broader stomach shadow with more vigorous peristalsis. Apparent constriction in buttermilk gastric tube is the imprint of gas in colon on stomach outline.

Barium milk contents are also invisible in the small intestine until the expiration of ten minutes which is the approximate time required for curdling milk in vitro at 98° F.

These distinctions of effect between milk and buttermilk meals are observed on the fluoroscopic screen but differences in gastric response thus induced can also be shown quantitatively. Fig. 1 illustrates this pictorially and Table I sets forth the distinction statistically. Average gastric shadow-width is 50 mm. for both milk and buttermilk, determined immediately below the Magenblase, but the gastric tube itself is distinctly broader after the buttermilk meal. In average vertical length the milk stomach is shorter and its shadow area but 92 per cent of that after buttermilk feeding.

TABLE I

1. COMPARATIVE GASTRIC DIMENSIONS: MILK AND BUTTERMILK SHADOWS

	NO.	AV. WIDTH	AV. HEIGHT	AV. AREA
Milk	31	50 mm.	206 mm.	11011 sq. mm.
Buttermilk	31	51	211	12150

2. INFLUENCE OF ORDER OF FEEDING: MILK AND BUTTERMILK

	AVERAGE AREA
Milk meal first	11372 sq. mm.
Milk meal following buttermilk	10673 sq. mm.
Buttermilk meal first	11937 sq. mm.
Buttermilk meal following milk	12377 sq. mm.

FACILITATION

To check our general observations recorded above we arrange the series so that half the number of students receive their milk meal first and their buttermilk meal an hour later. With the other half the order is reversed. Knowing that even trained and stable stomachs show a tendency to interference with the gastric reaction by obscure influences in the first examination of our regular two-day experimental session, we anticipated that a different picture might be obtained in the examinations of the second meal. In the second part of Table I the results of this inquiry are given. The milk meal, if given an hour after buttermilk produces a smaller shadow area than if administered first in the series. Buttermilk on the other hand gives a larger shadow when administered second. The characteristic features of outline are then intensified by exhibiting the vehicle after ingestion of a contrasting meal. This observation is so striking that it is well to accumulate all possible confirmatory evidence. Hence we shall take up the problem again after presenting the result of our studies with heat and cold. We give merely a summary of the influence of milk and buttermilk at the moment.

PASSAGE

Just as tone and peristalsis fluctuate independently the one of the other, so also peristalsis and passage are separate expressions of gastric activity. They may occur together or separately, and their association is more a coincidence than a co-partnership. Immediate passage often takes place as soon as the food reaches the pylorus and before peristalsis is set up. This initial passage lasts for but a few seconds but the fact of its occurrence even before the zones of irritability begin to pulsate demonstrates its essential independence of peristalsis. Rapid passage occurs in stomachs exhibiting only an occasional peristaltic wave, whereas there may be shimmer or even occasionally, vigorous peristalsis, with no evident passage. We purpose to extend our observations upon this problem but we are convinced that, while the cause of passage may be sought in gastric tone, it is not to be found simply in the peristaltic wave.

Both milk and buttermilk may be accompanied by immediate passage of barium through the pylorus. When this occurs it rarely lasts one minute, during which, however, the passage is usually continuous in contradistinction to the intermittent passage characteristic of later periods. The shadow of

immediate milk passage is light and often seen with difficulty. It may also be a thin stream whereas immediate buttermilk passage occurs in large dense shadows, often moniliform in silhouette though not discontinuous. This phenomenon appears in the notes as "passage in blobs." In the event of there being a residue of the previous contrasting meal in the stomach, ingestion of a new meal of buttermilk usually results in immediate ejection of the milk residuum or most of it. A meal of milk rarely brings about similar ejection of a buttermilk residue.

The ease with which passage is seen depends on the relation of pylorus to the duodenum. If the pyloric canal happens to be disposed in front of the second part of the duodenum observation of actual passage is often impossible. But if the pyloric canal is directed toward the right there are no difficulties in visualizing passage.

Down the second part of the duodenum and round the bend into the horizontal part the shadow can be followed, thin and smoke-like after a milk meal, dense and black and moniliform after buttermilk. The buttermilk-barium shadow more easily defines the cap and lingers in an evanescent cesspool at the bend between second and third portions. Duodenal peristalsis is easily seen after buttermilk: it is much more rapid than gastric peristalsis but the oscillating character of the shadow is present here as in the stomach. With every wave of peristalsis the cephalad level of the barium shadow shoots upward for an instant before dropping lower. In the second part of the duodenum this upward darting takes the form of streams or "prancing particles" which may even lose themselves in the cap shadow and so appear to be rejoining the main mass in the stomach.

There is no necessary relation between passage through the pylorus and gastric peristalsis. We constantly observe passage in the complete absence of peristalsis or we note well-marked peristalsis with no passage whatever. When peristalsis and passage occur in sequence we are inclined to attribute it to coincidence rather than to cause and effect.

In spite of the apparently greater size and density of shadow in buttermilk passage the stomach empties no more quickly than after a milk meal. This is an important observation which is in no way contradictory of the fact that some stomachs empty of milk or buttermilk alike much more rapidly than others. Such rapid-emptying stomachs usually evince evidence of the anxiety complex.

EFFECT OF QUANTITY

If, in place of a five-ounce meal, a sixteen-ounce meal is given, after the initial passage events occur more slowly. Peristalsis does not start after about two minutes on an average in a milk meal or continue without intermission from the beginning in a buttermilk meal. These features characterize the smaller amount. After a sixteen-ounce meal of milk it may be some time before real waves are seen and the buttermilk waves may be at their fastigium some forty minutes after ingestion. Apparently an over-distended organ does not show peristalsis well and it is only after the passage of a considerable volume of contents that the peristaltic wave develops effectively.

THE IRRITABLE STOMACH

It is well known that, in instances of duodenal disease, the stomach shows signs of irritability. We have had the opportunity of examining two definitely atypical stomachs. In one of these subjects there was evidence of occult haemorrhage in the alimentary canal and a clinical diagnosis of duodenal ulcer was definitely made. In the other there was no such diagnosis but the clinical symptoms were those typical of duodenal irritation. Both of these stomachs showed hypermotility equalizing milk and buttermilk responses so far as these relate to peristalsis but retaining the usual relative shadow dimensions and area. Both showed a marked reduction in emptying time. The latter of these stomachs we have examined afresh after subsidence of the symptoms and have observed that, although the stomach shows an active reaction to milk, it does not overstep the upper range of normal response. Further consideration of this most interesting phase must be left until we have had greater opportunities for observation.

STANDARD ROUTINE

In the practice of internal medicine, roentgenoscopic examination plays an increasingly important part in the diagnosis of gastro-intestinal disease. We are not ourselves concerned with such clinical conditions of the stomach as spasm, permanent cicatrization resulting in the hour-glass phenomenon, gastric ulcer as evidenced by the Nischen-symptom, or definite malformations of the shadow induced by foreign bodies, linitis, plastica, and cancer. These, when present, are obvious enough. We are interested, however, in the differential diagnosis of gastric response whether evidenced in dimensions or in motility. And we are greatly impressed with the effect of psychological influence upon gastric reactions. Our extended study of the student stomach has been planned with a view to securing greater definition of technique, and a finer discrimination in the diagnosis of functional disorder.

The first important result of our study is the contention that the usual examination of a starved stomach in a patient under considerable mental strain and even apprehension is not calculated to render easy the diagnosis of his disability. We believe that, wherever possible, a gastro-intestinal roentgenoscopic examination should be of a more extended type than that at present given, and that the results of two or three successive examinations are necessary for comparison before reaching a decision. We also believe that the conditions of the examination must be very carefully supervised and that no disturbing influence should be permitted to reach the patient at the time of examination.

The examination itself, we believe, should consist of two phases, and that standard five-ounce milk and buttermilk meals (four ounces vehicle, 33 gm. $BaSO_4$) should be given successively to a normally fed patient who has not tasted food or drunk water for two hours previously. The contrast between the gastric responses to the stimulation successively of milk and buttermilk will give a fairly definite indication of the gastric reaction pattern.

In our chapter on diagnostic meals we have spoken of cases of gastro-intestinal disorder in which the reaction pattern was exaggerated. We have

stated that our experience must be greatly amplified before reaching a definite conclusion on this matter. But we believe that a standard routine for gastrointestinal examination can be successfully evolved upon the lines which have just been laid down.

The reasonableness of this view seems to us apparent from our studies upon the medical students whose willing and loyal cooperation we gratefully acknowledge and without which this initial study and the further investigations which we propose to make could not possibly be undertaken.

SUMMARY

A milk meal results in a radiographic shadow relatively small in linear dimensions and in area. On roentgenoscopic examination the stomach is relatively inactive with peristaltic waves of small amplitude and low frequency. A buttermilk meal of equal volume results in a gastric shadow greater in its linear dimensions and area. But the stomach is more active and peristaltic waves are of greater frequency and amplitude. At the end of twenty minutes after either 5-ounce meal the stomach enters a neutral phase and shows a picture and a record of rather indifferent character so that it may be impossible to decide whether the meal has been of milk or of buttermilk.

These same meals of milk and buttermilk may be employed for diagnostic purposes and enable us to express an opinion upon the normal or abnormal motility of the stomach. But it is necessary to bear in mind that they can be so employed only after the subject has been trained to roentgenoscopic technique by several successive periods of examination.

Exaggeration of milk and buttermilk effects have been noted in definitely irritable stomachs. In these the milk meal is followed by a motility indistinguishable from that of buttermilk though the relative shadow dimensions and area are not modified.

ABSTRACT

In a trained stomach the ingestion of a 5-ounce milk-barium meal is followed by little elongation or lateral distension and by a peristaltic activity so gentle that it may be evinced by nothing more than a shimmer of the shadow outline of greater curvature. Passage occurs in light shadows like a puff of smoke and the duodenal cap may be indefinite in outline.

Under the same circumstances and in like conditions a 5-ounce buttermilk-barium meal is followed by greater elongation and lateral distension of gastric shadow so that the buttermilk shadow area is greater than the milk shadow area of the same stomach. Peristaltic waves are immediate, of considerable amplitude and massive in appearance. Passage occurs in dense moniliform shadows plainly followed through the duodenum.

These facts may be utilized in planning a standard technique of diagnostic meals.

REFERENCES

1. The Attainment of Reliability in Gastric Responses, J. Lab. & Clin. Med. **14**: 1017, 1929.
2. Stabilization of Gastric Reaction Patterns, J. Lab. & Clin. Med. **14**: 1165, 1929.

LABORATORY METHODS

THE CHEMICAL ASPECTS OF INTRAVENOUS GLUCOSE INJECTIONS*

By C. D. Ingersoll, Ph.D., Waukegan, Ill.

IN CONJUNCTION with some work on the preparation of glucose for intravenous injection a study has been made of the chemical aspects of the preparation and administration of intravenous glucose solution. Feeling that the results of this study will prove of interest to the medical profession, a résumé of the various chemical factors involved and the indicated technic is offered herewith.

PREPARATION OF THE SOLUTION

The aqueous solution of glucose is made by dissolving a high grade of glucose in pure distilled water and then sterilizing the solution obtained. It will be noted below that this final sterilization is a preventive measure following the use of sterile ingredients throughout and should not take the place of previous necessary precautions. The procedure is given in progressive steps after each of which appears a short discussion of the chemical points involved. In Table I will be found data for preparing 1 kilo (2.2 pounds) glucose solutions of 10 per cent, 20 per cent, 25 per cent and 50 per cent concentrations. The amount of solution prepared at one time may be varied as desired by multiplying the quantity given by an appropriate factor.

MATERIALS AND SOLUTIONS NECESSARY

Glucose C. P.—The glucose should be sterile and of approved high quality. It should contain no starch or dextrine and *no pathologically poisonous salts* such as those of lead or arsenic, which are sometimes used in industrial purifications. This also applies to oxalic acid and likewise to carbohydrate polymers of a carmel nature resulting from poorly controlled drying technic in manufacture. Either anhydrous or hydrous glucose may be used if allowance is made in weighing for the molecule of water of crystallization. For purposes of expressing the glucose content of one form in terms of the other, the following equivalents may be noted.

1 part by weight of Glucose Anhydrous—1.1 parts by weight of Glucose Hydrate
1 part by weight of Glucose Hydrate—0.91 parts by weight of Glucose Anhydrous

Distilled Water.—Tap water distilled without proper precautions may and usually does contain varying amounts of ammonia and nitrogenous compounds,

*From the Laboratories of the Pfanstiehl Chemical Co.
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organic matter, volatile acids, and even salts where the distillation is violent enough to cause entrainement of minute droplets. As traces of these compounds are liable to have a deleterious action on the patient when injected into the blood stream, it is highly desirable that they be eliminated. This may be accomplished by a convenient assembly of two distilling flasks in series. The first flask contains the raw (tap) water and a small amount of potassium permanganate (10 to 15 gm.) and the second flask about 100 gm. (or approximately 4 ounces) of barium hydroxide. A condenser is connected with the second flask. With the above apparatus chain, all of the organic matter is quickly oxidized in the first flask by the permanganate and for the most part is carried over, as carbon dioxide, through the barium hydroxide solution where it is entrained, together with any volatile acid present. An extremely high-purity sterile water is thus obtained.

It is further to be noted that only freshly distilled water should be used as bacterial growth has been found even in distilled water when stored for any length of time.

Data for intravenous glucose solutions of varying concentrations are given in Table I both in metric and English units and for the use of glucose anhydrous or glucose hydrate. It goes without saying that only glassware that has been washed and rinsed with distilled water should be employed.

TABLE I

GLUCOSE SOLUTION DATA

GLUCOSE CONCENTRATION DESIRED	GLUCOSE, ANHYDROUS				GLUCOSE, HYDRATE			
	METRIC SYSTEM		ENGLISH SYSTEM		METRIC SYSTEM		ENGLISH SYSTEM	
	GLUCOSE	WATER	GLUCOSE	WATER	GLUCOSE	WATER	GLUCOSE	WATER
			ounce	ounce			ounce	ounce
Per cent	gm.	c.c.	(av.)	liq.	gm.	c.c.	(av.)	liq.
10	100	900	3.525	30.42	110	890	3.88	30.10
20	200	800	7.05	27.05	220	780	7.76	26.35
25	250	750	8.82	25.35	275	725	9.70	24.50
30	300	700	10.58	23.65	330	670	11.63	22.65
50	500	500	17.63	16.90	550	450	19.39	15.22

The solution obtained should be clear and brilliant, although a slight sedimentation may occur on standing, the amount of which will depend on the quality of glucose used. There are also possibilities of a few dust particles. The prepared glucose solution should therefore be allowed to stand long enough to complete sedimentation, and then filtered through a high grade of filter paper which is first washed through thoroughly with distilled water. Low quality filter papers are liable to contain a small amount of soluble starch bodies and loose fibers. The washing of filter paper mentioned above is to eliminate possible sources of trouble from this source even in high grade papers.

The P_H should now be tested. Glucose is unstable in alkaline solution (i.e., those having a P_H greater than 7.0). On the other hand, the P_H of the blood stream is 7.3 to 7.4 (just on the alkaline side of neutrality) and is maintained there by the normal blood buffers.

It would appear the better part of good practice to adjust the P_H of the intravenous glucose solution to from 6.5 to 6.8 (just on the acid side of neutrality) using a small amount of sodium carbonate solution, or sodium phosphate

solution if necessary. The blood buffers will quickly absorb this slight acidity and maintain the normal blood P_H.

Immediately after filtration and adjustment of P_H, the solution is weighed into clean injection flasks, which have been rinsed with distilled water previous to drying. The flasks are then stoppered with corks that have been boiled in distilled water and the whole capped with a double layer of gauze between two double layers of filter paper; the cap is held on with a rubber band around the flask neck. After autoclaving for thirty minutes at 15 pounds, the flasks are removed, the corks pressed firmly into place and the paper gauze cap secured with several turns of adhesive tape.

The solutions should now be clear and brilliant and contain no foreign matter or insolubles of any kind.

STERILIZATION OF INJECTION APPARATUS

The sterilization of the needle and glass parts of the injection apparatus is a matter of such standard practice that they need not be entered into here. The principal item of interest at this point is the rubber tubing. Rubber tubing is a very convenient form of laboratory equipment but unfortunately carries certain hazards with its use which should be thoroughly understood in order to be adequately taken care of.

Due to the various compositions, textures and characteristics of the rubber tubings on the market only a high grade tubing should be used.

Due to the sulphur content, free talc and other components of the rubber, it should be boiled for fifteen to thirty minutes in 5 to 10 per cent sodium carbonate solution. This will thoroughly scour the rubber and is preferable to caustic soda as it will not be so violent in its action on the rubber. Following this, the tubing should be rinsed thoroughly by allowing a stream of hot distilled water to flow through it. (Tap water should never be used in the cleaning process as it contains many gelatinous and deleterious impurities which may temporarily adhere to the inner surface of the tubing and be later carried into the blood stream by the intravenous solution.)

The next step in washing is due to the fact that alkali has a decided tendency to cling to surfaces. This may be readily observed by dipping the hand in a soda ash solution and noting the difficulty with which the soda is rinsed off under the tap. It is suggested at this point therefore, to rinse the tubing with a ½ to 1 per cent solution of hydrochloric acid c. p. (made up with distilled water), and then to finally wash out by running distilled water through the tubing for fifteen to twenty minutes (test for completeness of washing with silver nitrate solution).

Other points of importance are the temperature of the glucose solution at the time of injection and the rate of injection. These points are primarily physiologic ones, however, and will not be considered here.

OBSERVATIONS ON THE ESTIMATION OF BLOOD SEDIMENTATION TIME*

By Robert A. Kilduffe,† M.D., Atlantic City, N. J.

THE recent revival of studies of the sedimentation rate of red blood cells has led naturally to a number of modifications of the original Linzenmeier technic, all of which have their advocates.

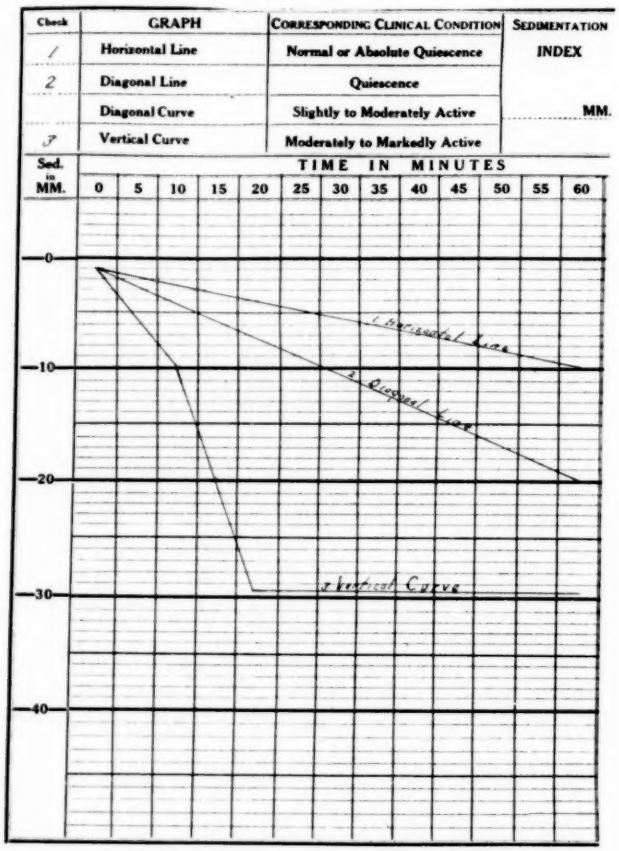

Chart 1.

It is more or less axiomatic that the simpler a method can be made without interference with accuracy, the more applicable it will be to the problems of clinical medicine, and many observers, therefore, have endeavored to simplify blood sedimentation technic.

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†Director, Laboratories, Atlantic City Hospital.
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It is not the purpose of this communication to discuss the methods devised for this test but to consider whether or not it is feasible to shorten the time allotted to the reaction and simplify the methods used for reporting the results.

That this is desirable may be hazarded from the multiplicity of methods used at present in reporting the test.

It is commonly accepted that there are two factors of clinical importance: (1) the velocity with which sedimentation occurs, which is very closely associated with the rapidity with which it is initiated; and (2) the degree to which the cells are finally sedimented.

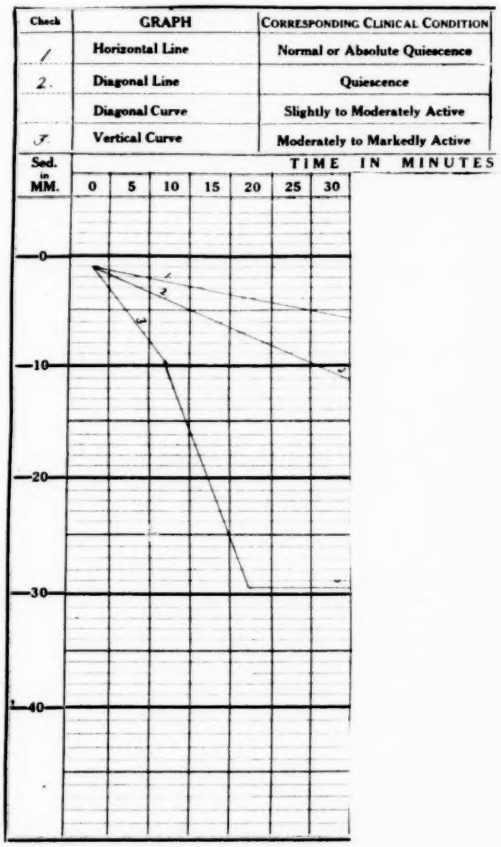

Chart 2.

Disregarding the variations introduced by variations in technic, it is apparent that the present methods of reporting sedimentation time depend upon the measurement of either distance or time. In other words, either the time is a fixed quantity, and the distance through which the cells have fallen during that period is recorded in millimeters, or the distance is a fixed quantity, and the time required for the cells to fall that distance is measured in minutes or hours.

As most commonly conducted, the reading of sedimentation tests requires a series of observations at short intervals conducted over a period of not less

than one and often several hours, during which the rate or velocity of the sedimentation is recorded at each time interval, the final record being concerned with the total volume occupied by the packed cells. The making of a single test requires, therefore, the undivided attention of the operator for at least one hour.

It is suggested in this communication that this time may be very materially shortened without interfering with the clinical significance of the procedure. The suggestion is based upon practical experience with various methods in several series and upon certain premises immediately following.

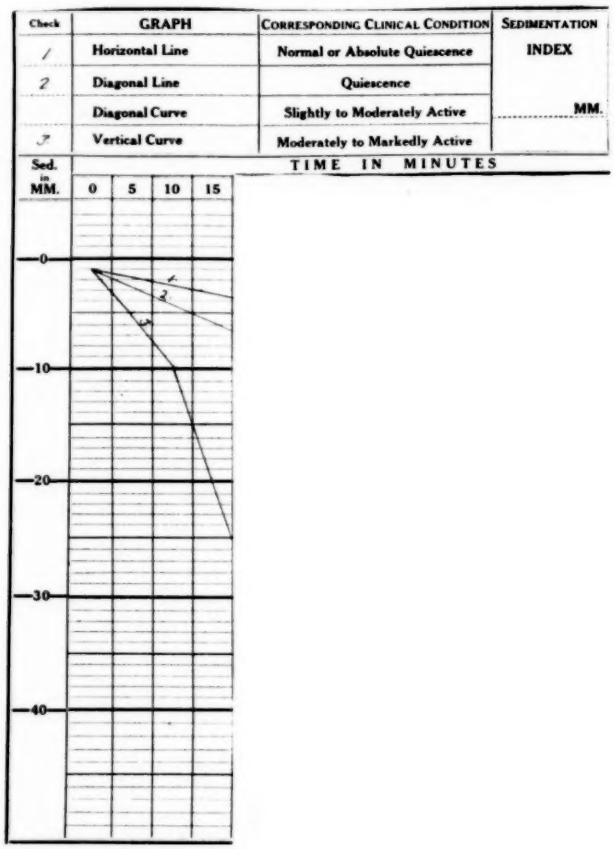

Chart 3.

In the first place, while it is not proposed to enter into the debatable field of the exact meaning of the test, it can be said that the majority of observers are agreed upon the fact that it possesses no *differential* value. In other words, while it quite definitely indicates *activity*, it does not definitely distinguish the kind of process which is active.

In the second place, while the velocity rate has been shown to exhibit certain variations when measured at five or ten minute intervals, it is dubious whether these variations are of any real clinical significance. It is true, also,

that regardless of the variations exhibited in any hourly period, the rapidity with which sedimentation *begins* is related to the velocity with which it continues. It is quite possible, therefore, within fifteen minutes of starting the test to determine whether or not the rate will be within normal limits, rapid, or very rapid.

Finally, there is in general a proportionate relation between the velocity of sedimentation and the final volume of packed cells.

On the basis of these observations, it is suggested, therefore, that equally as much information of clinical value can be obtained at the end of fifteen to thirty minutes as can be had after one hour, and that the reading could be reported after the shorter period without detracting from the usefulness of the test.

This may be brought out by comparing the two methods.

The method used in this laboratory is that of Cutler[1] whose charts are also used for report.

In Chart 1 are represented types of sedimentation tests conducted over a period of sixty minutes.

In Chart 2 are shown the same reactions terminated at thirty minutes.

In Chart 3 are shown the same reactions terminated at fifteen minutes.

A comparison of the charts suggests that the final character of the chart at sixty minutes is clearly indicated by the fifteen and thirty minute periods of the same chart. In other words, the type of the chart can be seen as easily and clearly at the shorter period as at the end of one hour.

[1]Cutler, J.: Graphic Presentation of Blood Sedimentation Test, Am. J. Med. Sc. **86**: 1972, 1926.

HOT PLATES FOR STAINING SPUTUM SLIDES*

By W. D. Stovall, M.D., and Vera Vincent, B.A., Madison, Wisc.

A HOT plate similar to Fig. 1 was adopted for use in the staining of sputum slides for the diagnosis of tuberculosis by this laboratory two years ago. Previous to this time we had been using a copper tank holding 25 slides as in Coplin jars. This was heated by a gas burner. The difficulty of satisfactorily cleaning this type of tank, especially the grooves which held the slides, with the ensuing danger of material from a positive slide being scraped off on the sides of the slots and later becoming attached to a negative slide, brought about a quest for a means of staining a large number of slides quickly with no danger of contamination of one slide by another.

The apparatus developed consists of two monel metal covered heating units each measuring 24½ by 3¾ by 2 inches. Monel metal was used to cover the units because it is resistant to the nitric acid in the decolorizer. The heating units proper were purchased from the Westinghouse Electric Company and are designated as Westinghouse Space Heater, S284220A. 110 volts, 500 watts.

*From the State Laboratory of Hygiene, Madison, Wisconsin.

These heaters are 24 inches long, and are covered the full length by the monel metal, which is attached to them on the under side. These two heaters are connected in series in order to achieve the proper temperature for steaming the stain. The current is controlled by a single-way switch attached to the frame of the appliance which can be operated from an ordinary light socket as it uses only 250 watts.

The units rest parallel to each other in a galvanized iron drain pan, the dimensions of which are 26 by 10 inches with 6½ inch legs. The outer edges of the pan are 1 inch deep and slope conically toward a center drain.

We found that the slides, when placed on the smooth monel metal covering the heating units, had a tendency to slip during the washing process and

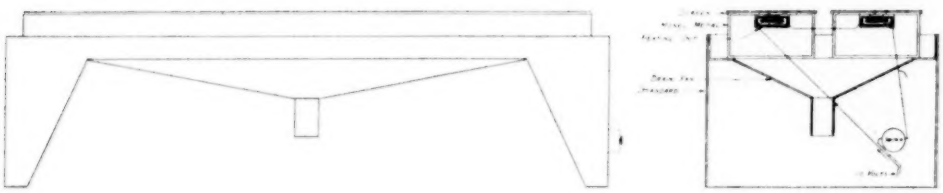

Fig. 1.

in order to counteract this difficulty two strips of wire screening were fitted over the metal, thereby affording a sufficiently rough surface to prevent any slipping.

The slides are laid on the screen 15 to the unit, which allows approximately ½ inch between slides. They are stained for fifteen minutes, ten minutes with the heat turned on and five after it has been turned off. This gives the slides a chance to cool before the decolorizer is applied, a desirable precaution since we feel that there is a possibility that the slides decolorize too rapidly while hot.

The apparatus is placed near a sink which permits the complete staining and decolorizing process to be carried out on the plates. The stains and decolorizer are applied from dropper bottles permitting a small stream flow of the reagent. A rubber hose, attached to a near-by faucet, is used for washing the slides, and the waste water is carried to the sink by a hose connected to the under drain.

A NEW METHOD FOR THE DETERMINATION OF CHLORIDES IN THE BLOOD*

By E. Földes, M.D., and H. Tauber, Ph.D., Brooklyn, N. Y.

IN THE following we intend to describe a method for the determination of chlorides in the blood. The method is based on the principle introduced in chemistry by Mohr: the titration of chlorides with silver nitrate in a neutral solution, using potassium bichromate as an indicator.

The necessary solutions are the following: 0.2905 per cent $AgNO_3$ solution, preserved in a brown bottle; 2.5 per cent Na_2CO_3 solution; 10 per cent $K_2Cr_2O_7$ solution; 1 per cent alcoholic phenolphthalein solution.

All reagents should be especially tested for the presence of chlorides.

Ten c.c. of the protein free blood filtrate prepared in the usual manner according to Folin-Wu, are measured with a pipette in an Erlenmeyer flask. After having added 2 to 3 drops of the phenolphthalein solution, the blood filtrate is neutralized, drop by drop with the sodium carbonate solution until a faint red color appears. Three drops of the potassium bichromate solution, being used as an indicator at the titration, are now added, whereupon the red color disappears and is replaced by a color similar to that of amber wine. The titration is performed with the silver nitrate solution from a burette. During the titration the blood filtrate gradually assumes the greenish yellow color of lime, and the end point is marked by the changing of this to a brownish color which does not disappear even after vigorous shaking. The change of the colors at the end point is very sharp and a great accuracy of the titration can be secured after a short training.

One c.c. of the above given silver nitrate solution being equivalent to 1 mg. NaCl, the number of c.c. of $AgNO_3$ solution used in the titration indicates the chlorides present in the blood filtrate as NaCl, in mg. As furthermore 10 c.c. of the protein free blood filtrate represents 1 c.c. of the original blood, the multiplication by 100 of the number of c.c. silver nitrate solution consumed during the titration will indicate the chlorides as NaCl in mg. in 100 c.c. blood.

The question as to whether the results obtained by the method described conform with the actual quantity of chlorides present in the blood was examined by two series of checks. In the first series, we measured in each of 6 Erlenmeyer flasks 10 c.c. of protein free filtrate of the same blood. To each of two of these filtrates we added 3 c.c. and to each of other two filtrates 5 c.c. of a NaCl solution of exactly 1 per mille, and then we determined by our method the chlorides in all 6 filtrates. The results obtained (calculated for 100 c.c. blood) were as follows: 500 mg. and 493 mg., respectively, in the original filtrates; 797 mg. and 810 mg., respectively, in the filtrates to which 3 mg. of NaCl was given; and 998 mg. and 1003 mg., respectively, in the filtrates to

*From the Department of Laboratories, United Israel Zion Hospital, Brooklyn, N. Y.
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which 5 mg. of NaCl was added. This makes it apparent that we recovered with great accuracy the NaCl added to the filtrates.

In another series, we determined the chlorides in the blood of a number of different patients first by our method and then by Volhard's method as described by Whitehorn,* and compared the results obtained (see Table I).

TABLE I

NO.	CHLORIDES AS NaCl, MG. IN 100 C.C. OF BLOOD BY METHOD			
	DESCRIBED		OF WHITEHORN	
	FIGURES OBTAINED	MEAN	FIGURES OBTAINED	MEAN
1	518 514	516	500 505	502
2	443 451	447	447 433	440
3	490 487	488	476 476	476
4	558 565	561	548 561	554
5	530 532	531	515 527	521
6	533 544	538	533 525	529
7	463 470	466	469 472	470
8	520 515	517	510 508	509
9	475 482	478	470 480	475
10	493 504	498	495 494	494
11	455 454	454	463 458	460
12	461 463	462	464 472	468
13	530 539	534	515 531	523
14	508 508	508	493 501	497
15	527 516	521	510 515	512
16	552 555	553	550 560	555
17	460 460	460	455 460	457
18	592 606	599	584 594	589
19	512 511	511	485 495	490
20	545 550	547	540 545	542

The table shows a great conformity between the results obtained by our method and that of Whitehorn. Taking the latter as a basis for comparison, the differences between the means obtained by the two methods on the same specimen of blood varied between −1.3 and +4.5 per cent. The average figures of all 20 specimens of blood were 509 mg. with our method and 503 mg. with that of Whitehorn, giving an average difference of +1.2 per cent.

Considering the differences between the two control determinations performed by the same method on each specimen of blood, the highest observed

*Whitehorn: J. Biol. Chem. 45: 449, 1921.

difference was 2.3 per cent with our method (No. 18) and 3 per cent with Whitehorn's method (No. 13). The average difference between the two control determinations was 1 per cent with our method and 1.5 per cent with Whitehorn's method.

The close conformity between the results obtained by our method and that of Whitehorn, but particularly the fact that we recovered exactly the amount of NaCl added to the blood (as shown above) indicates that the method described is one of great accuracy. It offers furthermore distinct advantages over other means of determining chlorides in the blood, one of which is the sharp end point of the titration, as is also indicated by the smallness of the extreme and average difference between the control determinations of the same specimen of blood. The method is also of the greatest simplicity, inasmuch as in contradistinction to other methods, it requires only the silver nitrate solution for the titration. Thus the preparation of a second quantitative solution for titration and the frequent painstaking adjustment of the two solutions, as required, for instance, by Whitehorn's method (silver nitrate and potassium sulphocyanate), can be eliminated.

SUMMARY

A method for the determination of chlorides in the blood is described. In addition to an accuracy, comparable to that of the best methods, a distinct advantage of the method described is its greater simplicity.

We wish to express our gratitude to Dr. M. Goldzieher, Director of the Laboratories, for his kind interest in this work and for the assistance rendered.

HEATING OF SERA IN THE KAHN TEST*

By Nathan Nagle, A.B., and Martha Monell, St. Louis, Mo.

A STANDARD technic of the Kahn test has been developed as an outgrowth of certain principles which underlie the phenomenon of precipitation in syphilis. Every step in this technic must be rigidly followed to do the test correctly. Any deviation from the method evolved by Kahn, changes the precipitation test sufficiently that it is a modification of the Kahn test and not the standard method. It is unfair to the standard Kahn test when it is performed with antigen that is not standard; with shaking periods varying from the standard; with the reading of the test made after overnight incubation; with the serum control omitted and with the inactivation time or temperature changed to meet the mood or fancy of the technician. Each step in the Kahn procedure is vital to the test. Therefore, it is necessary to carry it out in accordance with requirements as prescribed by the author to do justice, both to the test and to the physician and patient.

It is true that the Kahn system has not reached the acme of perfection. It has been devised as a practical test in the laboratory diagnosis of syphilis. As such, it is a very useful aid to the clinician. To the laboratory worker it offers a very convenient medium for studying many problems relating to the serologic diagnosis of syphilis. The comparative simplicity and practicability of the test has stimulated workers in studying the various phases of the precipitation phenomenon. Using the Kahn test in an experimental manner should be encouraged. It should be cautioned, however, that the standard test should be used in all of its details when employed as a routine diagnostic procedure.

One vital step in the Kahn technic is the heating of sera for thirty minutes at 56° C. The end-results of the Kahn test are materially changed if any other time or temperature is used. It is possible to change the reaction of a positive serum from four-plus to negative by manipulating the temperature in the heating process. Usually sera giving four-plus reactions with the standard test are negative when the test is made with unheated sera. Negative results may also be obtained if positive sera are heated at 60° C. or 62° C. for thirty minutes. A change of 2° from the standard temperature over a thirty-minute period may change the reaction of positive sera. Increasing the temperature above 56° C. may also account for some atypical reactions. Some sera giving a ++++ ++++ ++++ in the standard test, may give: ++++ ++++ +, ++++ +++ ++, or ++ ++ ++ when the sera are heated at 60° C. or 62° C. for thirty minutes or less.

Experiments were conducted in this laboratory with the Kahn test on heating sera at various temperatures and for various lengths of time to ascer-

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tain whether it was possible to select a more suitable temperature than 56° C. for use in the routine set-up of the Kahn test. Experiments were also made to find whether a period shorter than thirty minutes at a temperature higher than 56° C. would give good results in this test. In all of this work the standard procedure was always used to check the experimental temperatures and periods of time.

Eighty-seven sera were heated at 60° C. and 42 at 62° C. for thirty minutes and compared to the standard method. A summary of the results is shown in Tables I and II. An analysis of these tables shows clearly that heating sera in this manner tends to inhibit precipitation, especially in sera giving weak reactions. Weak reactions in the Kahn test are very significant in the diagnosis of syphilis and in treated cases as was observed by different workers and in this laboratory.[1]

TABLE I

NO. OF SERA	8	4	1	1	1	1	1	1	1	1	3	1	63
Standard 56° C.–30 min.	++++	++++	++++	++++	++++	+++	+++	++	+	+	+	–	–
60° C.–30 min.	++++	+++	++	+	–	++	–	+	+	±	–	+	–

TABLE II

NO. OF SERA	7	3	1	1	1	1	1	27
Standard 56° C.–30 min.	++++	++++	++++	+++	++	+	–	–
62° C.–30 min.	++++	+++	–	–	±	–	±	–

Judging from the above results it is evident that heating sera at a higher temperature than 56° C. for thirty minutes does not improve the Kahn reaction. Accordingly, tests were made on 112 sera heated at 62° C. and 175 heated at 60° C. for twenty minutes. As shown by Tables III and IV the standard method gives better results.

TABLE III

NO. OF SERA	12	3	1	4	1	2	1	2	1	1	1	83
Standard 56° C.–30 min.	++++	+++	++++	++++	+++	+++	+++	+++	+	+	+	–
62° C.–20 min.	++++	+++	+	–	+++	++	+	–	+	±	–	–

TABLE IV

NO. OF SERA	6	2	1	2	1	2	4	1	1	2	1	1	1	150
Standard 56° C.—30 min.	++++	++++	++++	+++	+++	+++	+++	++	++	+	+	–	–	–
60° C.—20 min.	++++	+++	–	++	+	++++	+++	+++	++	+	–	++	±	–

One hundred and thirty-eight sera were heated at 62° C. for fifteen minutes. Table V shows that these reactions are definitely weaker than the standard test.

TABLE V

NO. OF SERA	23	4	1	1	1	2	1	1	1	1	2	100
Standard 56° C.—30 min.	++++	++++	++++	+++	+++	+++	+++	++	+	+	±	−
62° C.—15 min.	++++	+++	−	+++	++	+	−	±	+++	−	−	−

Two hundred and twenty-three specimens were tested after heating at 60° C. for fifteen minutes. The results obtained in this group parallel the standard test very closely. Even so, the small number of tests made at this temperature and time is not conclusive evidence that it is more suitable than the standard technic. It would seem that in emergency where the saving of fifteen minutes would be of some importance, it would be safe to use this technic. Table VI shows the results in this series. It will be noticed that even weak reacting sera check very closely with the standard test.

TABLE VI

NO. OF SERA	48	3	7	1	1	2	3	1	1	1	155
Standard 56° C.—30 min.	++++	++++	+++	+++	++	++	+	±	±	−	−
60° C.—15 min.	++++	+++	+++	++++	+++	++	+	−	+	±	−

As shown by Table VI heating of sera at 60° C. for fifteen minutes approximates the standard method very closely in regard to sensitiveness. It is also seen that heating of sera in this manner is slightly more destructive to the syphilitic reagin than is the standard procedure. Accordingly, 204 sera were heated at 60° C. for ten minutes. Even heating sera for this short length of time is sufficient to alter the test so that the reactions are less sensitive than the standard. Table VII gives a comparison of these results.

TABLE VII

NO. OF SERA	35	2	1	3	2	1	2	1	1	2	1	1	1	151
Standard 56° C.—30 min.	++++	++++	+++	+++	+++	++	++	++	++	+	+	+	+	−
60° C.—10 min.	++++	+++	+++	+++	++	+++	++	+	−	+	+++	±	−	−

Experiments in which sera were heated at temperatures less than 56° C. for different periods of time were not undertaken because of the work done by Kahn. Using unheated sera and portions of the same sera heated at 56° C. for five, ten, twenty, thirty and sixty minutes showed that better results were obtained at the thirty-minute period than at the shorter time periods. Heating of sera for sixty minutes resulted in slightly more sensitive reactions than obtained at the thirty-minute period. Other experiments conducted at the same temperature but using longer heating periods showed that the syphilitic reagin began to be destroyed only after ninety minutes of heating. Kahn states, ". . . that the more potent the serum the shorter the inactivation required. The very strongly positive sera apparently did not

require inactivation. Sera of somewhat lesser potency required no more than five minutes of inactivation, while still weaker sera apparently required a minimum of thirty minutes heating.''[2]

Sera are heated in the complement-fixation tests for the purpose of destroying native complement. Sera are heated in the Kahn test because experiments conducted have shown that this process enhances the precipitation phenomenon. It is not known just what heat does to sera, but it is known that certain temperatures enhance the specific reacting substance while other temperatures destroy this substance. It may be conjectured that heat changes a raw serum from a stable to an unstable form which enables the specific reacting substance in the serum to unite more readily with antigen units. Whatever the explanation may be, it is definitely known that to secure the best results with the Kahn test it is necessary to heat sera at 56° C. for thirty minutes.[3] Any deviation from this procedure results in a Kahn test that is less accurate and which deviates materially from the standard test.

CONCLUSIONS

The standard heating period of sera for use in the Kahn test is thirty minutes at 56° C. Experiments were conducted to find a more suitable temperature than 56° C. and a period shorter than thirty minutes. It was found that heating sera at higher temperatures than 56° C. for thirty minutes and for shorter time periods was inhibitory to the precipitation phenomenon and was therefore unsuitable for use in the Kahn test.

This study was aided by the support and helpful suggestions given by Dr. Joseph C. Willett, Chief Bacteriologist.

REFERENCES

1. Willett, J. C., and Nagle, N.: The Kahn Test in Primary Syphilis, J. A. M. A. **90**: 1189, April 14, 1928.
2. Kahn, R. L.: Serum Diagnosis of Syphilis by Precipitation, 1925, Baltimore, Williams and Wilkins Company.
3. Kahn, R. L.: The Kahn Test—A Practical Guide, 1928, Baltimore, Williams and Wilkins Company.

RELIABILITY OF THE GONOCOCCUS FIXATION TEST*

By Thomas G. Hull, Ph.D., Chester Garwood, and Nell Hall,
Springfield, Ill.

THERE are two factors to be considered in the application of the complement-fixation test to gonorrhea, first, from a laboratory standpoint, and second, from a clinical standpoint.

Numerous reports of laboratory investigations have found their way into the scientific literature since this test was first used in 1906. During that period considerable progress has been made not only in the sensitivity and reliability of complement-fixation in general, but also in the application of this procedure to gonorrhea when used with a gonococcus antigen.

The proper interpretation of the results of the test by the clinician is necessary. In some instances unfamiliarity with the shortcomings of the procedure has led to erroneous conclusions, in other cases an undue enthusiasm in favor of or against the test has influenced the interpretation. It was our endeavor recently to discover the attitude of clinicians for whom we had been making the test. A preliminary survey showed that some had found it, in their experience, reliable and of value to them in determining the diagnosis; others had had an opposite experience while a few seemed loath to say anything against a test in which there might possibly be some good. On the whole the attitude of clinicians in Illinois was about the same as that expressed in the "Principles and Suggestions Accepted at the January Conference of Venereal Disease Clinicians" in 1927, "the complement-fixation test for gonorrhea is reported by certain clinicians as a satisfactory diagnostic test, to aid in the diagnosis of chronic gonorrhea especially in cases with suspicious lesions or discharges and negative smears. Other reports indicate it is of doubtful value."

The investigation was continued with the results reported in the following paragraphs.

THE ANTIGEN

Antigens for the gonococcus fixation test were obtained from four different sources and tested in paralled on the same specimens of serum. Antigen C was from a research laboratory, the others were from commercial biologic houses. In response to a request for information, the following was submitted as to the method of preparation of each antigen.

Antigen A.—"The gonococcus antigen is prepared from eleven different strains of the gonococcus from the cultures of which the growth is removed with sterile salt solution and placed in the ice box where it is frequently shaken for a period of twenty-four hours. The autolyzed material is then diluted to

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the proper volume with sterile salt solution and preserved with 0.5 per cent trieresol, after which it is again shaken and placed in the ice box for twenty-four hours before testing."

Antigen B.—"The antigen is a bacterial suspension prepared from four Torrey strains."

Antigen C.—An autolized extract in normal salt solution made from polyvalent strains of gonococci or a more generalized single strain such as gonococcus No. 34 of Torrey.

Antigen D.—"The gonococcus antigen consists of an emulsion of dehydrated, defatted gonococci in 50 per cent glycerol. It is prepared from the two Torrey strains Nos. 34 and 42, which Dr. Torrey recommends as having the best antigenic properties. The antigen is made according to Torrey's method, except that instead of having the finished product in physiologic salt solution, we use 50 per cent glycerol which acts as an excellent preservative and prevents loss of antigenic properties."

The results of the parallel tests are given in Table I. It would seem that the antigen containing the most number of strains of gonococci gave the largest number of fixations.

TABLE I

FOUR ANTIGENS RUN IN PARALLEL ON 100 SERUMS

	ANTIGEN A	ANTIGEN B	ANTIGEN C	ANTIGEN D
Positive	27	6	16	25
Slightly positive	10	3	5	5
Negative	63	90	79	70
Anticomplementary	0	1	0	0

THE TEMPERATURE OF FIXATION

It has been the experience of numerous workers that the ice box method of fixation for a period of four hours to overnight gives the most sensitive results. Comparative tests were made with two antigens by each method. The results, given in Table II emphasize the greatly increased sensitivity by using the prolonged fixation at 6° to 8° C.

TABLE II

TWO ANTIGENS RUN BY WATER-BATH AND ICE BOX FIXATION METHODS ON 74 SPECIMENS

	WATER-BATH 30 MINUTES	ICE BOX OVERNIGHT
Antigen A—		
Positive	8	22
Slightly positive	3	2
Negative	63	48
Anticomplementary	0	2
Antigen D—		
Positive	6	14
Slightly positive	5	6
Negative	63	52
Anticomplementary	0	2

THE CLINICAL VALUE OF THE TEST

Clinical histories were submitted with 1170 blood specimens from 822 patients giving age, sex, and clinical diagnosis together with any other pertinent facts. Age was apparently no factor. The specimens were equally divided

TABLE III

THE GONOCOCCUS FIXATION TEST IN 822 CASES

	POSITIVE TEST	SLIGHTLY POSITIVE TEST	NEGATIVE TEST
Acute gonorrhea	30	4	26
Subacute gonorrhea	16	2	13
Chronic gonorrhea	63	26	39
Suspected gonorrhea, diagnosis not complete	11	0	0
Old history of gonorrhea	8	0	0
"Cured" cases of gonorrhea	0	2	88
Syphilis	17	3	65
Tuberculosis	1	1	13
Other miscellaneous affections	9	4	90
"Not gonorrhea"	20	15	256
	175	57	590

TABLE IV

RESULTS TABULATED ACCORDING TO SEX

	FIXATION TEST	MALE	FEMALE
Acute gonorrhea	positive	24	6
	slightly positive	4	0
	negative	20	6
Subacute gonorrhea	positive	8	8
	slightly positive	1	1
	negative	7	6
Chronic gonorrhea	positive	40	23
	slightly positive	15	11
	negative	26	13
Suspected gonorrhea (diagnosis not complete)	positive	6	5
Old history of gonorrhea	positive	8	2
"Cured" cases of gonorrhea	slightly positive	2	0
	negative	14	74
Syphilis	positive	11	6
	slightly positive	2	1
	negative	37	28
Tuberculosis	positive	1	0
	slightly positive	0	1
	negative	5	7
Other affections	positive	4	5
	slightly positive	1	3
	negative	44	47
"Not gonorrhea"	positive	13	7
	slightly positive	6	9
	negative	115	135
		414	404

between male and female. Among tests from the 822 patients there were 175 positive reactions, 57 slightly positive and 590 negative (Table III).

Acute Gonorrhea.—Sixty specimens showed 26 negative reactions the majority of which were in the first two weeks of the disease and none later than

TABLE V

INTERVAL BETWEEN DATE OF FIRST SYMPTOMS AND DATE OF TEST

	ACUTE GONORRHEA		
	POSITIVE TESTS	SLIGHTLY POSITIVE	NEGATIVE
1 week or less	1	0	8
2 weeks	5	0	7
3 "	3	0	2
4 "	3	1	2
5 "	3	0	2
6 "	1	0	1
7 "	0	0	
2 months	2	1	
3 "	1	0	
4 "	1	0	
5 "	1		
7 "	1	0	
8 "	1	0	
9 "	1	1	
1 year	0		
2 years	1		

TABLE VI

INTERVAL BETWEEN FIRST SYMPTOMS AND DATE OF TEST

	SUBACUTE GONORRHEA		
	POSITIVE TESTS	SLIGHTLY POSITIVE TESTS	NEGATIVE TESTS
1 month or less	1		3
2 months	5	–	1
4 "	1	–	1
6 "	3	–	0
8 "	1	–	1
9 "	1	–	–
10 "	–	1	–
1 year	–	–	1

TABLE VII

INTERVAL BETWEEN DATE OF FIRST SYMPTOMS AND DATE OF TEST

	CHRONIC GONORRHEA		
	POSITIVE TESTS	SLIGHTLY POSITIVE TESTS	NEGATIVE
1 month or less	1	1	1
2 months	2	0	0
3 "	2	1	2
4 "	1	0	0
5 "	3	0	0
6 "	6	1	3
7 "	0	0	
8 "	0	0	
9 "	1	1	
1 year	10	6	4
2 years	13	1	10
3 "	6	3	4
4 to 7 years	2	0	3
8 to 10 years	3	1	1
11 years and more	2	4	2

the sixth week (Table V). The positive reactions grouped themselves mostly in the second to the sixth week with a few scattered ones later on. All but 12 of the specimens from cases of acute gonorrhea were from males (Table IV).

Subacute Gonorrhea.—Thirty-one specimens showed 13 negative reactions mostly in the first month. The positive reactions on the other hand were mostly in the second to the sixth months (Table VI). The same number of specimens were received from male and female (Table IV).

Chronic Gonorrhea.—One hundred thirty-one specimens showed 39 negative reactions. The time intervals for most of the specimens were one to eleven years or more after the date of first symptoms (Table VII). There were 81 specimens from males of which 32 per cent were negative and 67 specimens from females of which 19 per cent were negative (Table IV).

"Cured" Cases of Gonorrhea.—Ninety specimens showed 2 slightly positive reactions and the rest negative. Many of these were at an institution for women where careful observations had been made over a long period of time. Smears and fixation tests that formerly were positive had become negative and remained so consistently.

Conditions Other Than Gonorrhea.—There were specimens of blood sent in from 494 miscellaneous conditions diagnosed other than gonorrhea. Of

TABLE VIII

MISCELLANEOUS CONDITIONS WHICH GAVE NEGATIVE GONOCOCCUS FIXATION REACTIONS

Arthritis	14
Duodenal ulcer	6
Focal infection from tonsil	4
Pregnancy	4
Carcinoma	4
Neurosis	4
Neurasthenia	3
Hypertension	3
Cystitis	3
Hyperthyroidism	3
Nephritis	2
Endocarditis	2
Furunculosis	2
Appendicitis	2
Influenza	2

One each of the following—cardiac hypertrophy, hypotension, polyneuritis, tumor, colitis, vaginitis, chronic rhinitis, pyelitis, pyelocystitis, cholecystitis, oophoritis, hemorrhoids, bronchial asthma, aneurysm of aorta, gastritis, chronic bronchitis, acute eczema.

TABLE IX

REPEATED NEGATIVE FIXATION TESTS IN 79 FEMALE CASES DIAGNOSED "NOT GONORRHEA"

16	persons	2	negative tests
5	"	3	" "
10	"	4	" "
9	"	5	" "
13	"	6	" "
11	"	7	" "
5	"	8	" "
4	"	9	" "
3	"	10	" "
2	"	11	" "
1	"	12	" "

those, 424 were negative, 85 per cent. Syphilis gave 20 positive or slightly positive reactions out of a total of 85 specimens; tuberculosis, 2 positive or slightly positive reactions out of 15; other miscellaneous affections gave 13 such reactions as follows: rheumatism, neurosis, appendicitis, epilepsy, boils, goiter, sinusitis, hemiplegia one each, pregnancy 2, and adenitis 3. The miscellaneous conditions giving 90 negative reactions are listed in Table IX. There were 291 specimens reported from patients diagnosed "not gonorrhea" of which 6 per cent gave positive reactions, 5 per cent partial reactions, and 89 per cent negative reactions.

The constancy of negative reaction in persons not having gonorrhea is shown in 79 female cases at an institution where two or more tests were repeated on the same individuals (Table IX). Four hundred twenty-seven tests repeated on 79 persons gave uniformly negative reactions.

COMMENT

The shortcomings of this form of a statistical study is fully realized. It is difficult enough to obtain accurate diagnosis on a large number of cases at a clinic where the work is carefully supervised. There are bound to be discrepancies, therefore, in diagnoses made by several hundred different physicians some of whom perhaps are not specialists in diagnosing gonorrhea. While the results of the study must be made in general terms, they are sufficiently conclusive to be of interest.

SUMMARY

1. The gonococcus fixation test should be made with an antigen composed of as many strains of gonococci as possible. Some antigens on the market contain as few as two strains.

2. The value of the ice box method of fixation overnight is fully confirmed.

3. Cases of acute and subacute gonorrhea show negative reactions early in the disease, but tend to become positive later; cases of chronic gonorrhea show positive fixation reactions in about 70 per cent of cases.

4. A small percentage of cases which have not been diagnosed gonorrhea give positive reactions.

5. Persons not having gonorrhea that do not give a positive test at one time probably will not give a positive test at a later time. Four hundred and twenty-seven tests repeated on 79 individuals were uniformly negative.

REFERENCE

1. Report of a Conference of Venereal Disease Control Officers and Clinicians, held in New York Jan. 20, 1927, Venereal Disease Information, U. S. Public Health Service **8**: 72, 1927.

AN EASILY CONSTRUCTED SLIDE RULE FOR CALCULATING THE DATE ON WHICH A GIVEN INTERVAL IN DAYS WILL FALL

By Theodore S. Moise, M.D.,* Bangor, Maine

THE slide rule, herewith described is an easily made instrument that may be used for saving time and labor in calculating the dates on which a known time interval in days will fall. The author has used it with great benefit in calculating the dates of termination of a large series of experiments, running for definite time intervals.

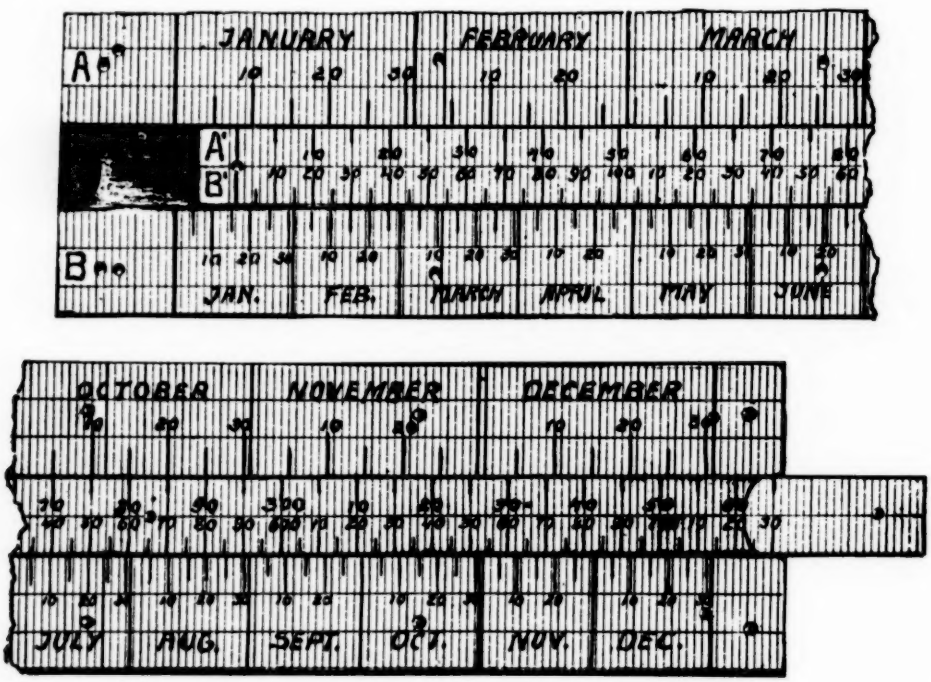

Fig. 1.

The graduations on the slide rule are measures of length representing increments of time in days. The scales seen in Fig. 1 were made on Keuffel and Esser, Standard Profile Paper 4 by 20 to the inch. On scale *A*, one division ($\frac{1}{20}$ inch) is taken as one day, and a linear calendar† was drawn. The end of each month is represented by lines extending completely across the scale. On scale *A'* the same unit ($\frac{1}{20}$ inch) represents a day with three hundred and

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†A correction for February 29 (omitted from scales) is easily made when necessary.

sixty-five days drawn in a linear manner independent of the calendar months. On scales *B* and *B'*, a similar representation of two years is made with each division representing two days. Scales *A'* and *B'* are on the sliding portion of the rule.

A calculation is made in the following manner: Example: Fifty-six days after January 8. The zero mark on scale *A'* is set opposite January 8. On scale *A'* (Fig. 1), 56 is then located and the reading March 5 is made above on scale *A*. The rule may be set to make other similar calculations. In practice, scales *A* and *A'* are more useful than scales *B* and *B'* as an accurate reading is more easily made with the larger unit. In calculating the date after an interval extending from the latter part of the year beyond January 1 of the following year, the reading may be made as follows: Example: Fifty-six days after December 5. Opposite December 5 on scale *A*, set 365 on scale *A'*

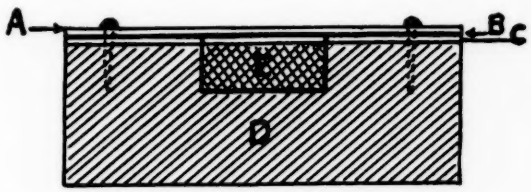

Fig. 2.

Turn the eye to the left and read the date on scale *A* just above 56 on scale *A'* Calculations of more than three hundred and sixty-five days may be made on scales *B* and *B'*.

The rule is ¾ by 2 by 19½ inches. Scales *A* and *B* are ¾ inch wide. The sliding portion of the rule including scales *A'* and *B'* is ½ inch wide. In order to prevent stretching and shrinking of the profile paper (with consequent introduction of an error) the device of mounting* a two inch strip of profile paper on heavy drawing paper was adopted. The scales were drawn on this mounted paper before being cut to the proper size. The scales were fastened to the rule by small tacks. The construction of the rule is shown in Fig. 2. *A* is a thin transparent celluloid cover. *B* is the profile paper on which the scales are drawn. *C* is a piece of heavy drawing paper. *D* is the body and *E* is the sliding portion of the rule.

*Kodak dry mounting tissue was used.

A STUDY OF HEMOGLOBIN METHODS*

By M. Karshan, Ph.D., and R. G. Freeman, Jr., M.D., Staten Island, N. Y.

MANY clinical methods for the determination of hemoglobin have been proposed. At the present time, with the wide variety of methods in use, many of them of questionable accuracy, it is impossible to compare the results of one worker with those of another or to evaluate the results of a single piece of work because of the inaccuracy of the method employed. The purpose of the work to be described in this paper is to determine the accuracy of a simple clinical method by comparison with a standard method of recognized accuracy.

From the review of Lindsay, Rice and Selinger[1] it appears that of the clinical methods, those of Newcomer[2] and of Cohen and Smith[3] are the most accurate. As for the Talquist, the Dare and Sahli methods, their inaccuracy has been repeatedly pointed out.[1, 6, 14]

The Newcomer, and the Cohen and Smith methods are based on the same principle, namely, the conversion of a definite quantity of blood to acid hematin by means of dilute hydrochloric acid. The color thus developed is compared with a standard, which in the Newcomer method is a "high transmission yellow" semaphore glass disk. In the Cohen and Smith procedure a standard acid hematin solution is used, and the authors claim an accuracy to within 2 per cent in the hands of inexperienced workers and a margin of error of 1 per cent or less by experienced workers. Shortly after the publication of the method of Cohen and Smith, Robscheit[4] published a method almost identical to that of the former authors. Comparing this with the Palmer[5] method excellent checks were obtained.

We chose to investigate the Cohen and Smith method, incorporating the modifications recommended by Oser[8] in so far as the quantity of blood to be analyzed is concerned. Cohen and Smith use 0.02 c.c. of blood in 6 c.c. of 0.1 N HCl, whereas Oser uses 0.05 c.c. of blood in 10 c.c. of 0.1 N HCl. A deeper color is thus obtained, and the larger volume makes for greater convenience and accuracy in making the color comparison.

Several attempts were made to prepare the standard hematin solution from oxalated blood. In every instance solutions containing a considerable quantity of a coarse suspension were obtained. This did not occur when the original procedure of Cohen and Smith using defibrinated blood was employed. The standard was prepared by determining the hemoglobin content of the blood by the Van Slyke and Neill[9] oxygen capacity method and then diluting with 0.1 N HCl to give a 3 per cent solution of acid hematin.† From this stock

*From Seaside Hospital, St. Johns Guild, Staten Island, N. Y.
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†If the hemoglobin concentration is 14.2 per cent, dilute $\frac{20 \times 15}{14.2}$ or 21.1 c.c. to 100 c.c. with 0.1 N HCl.

solution, a dilute acid hematin solution was prepared for color comparison with the unknown, by diluting 5 c.c. to 200 c.c. with 0.1 N HCl.[8]

The Van Slyke[7, 9, 12] oxygen capacity methods, while standard, present many technical difficulties and necessitate considerable practice. Wong[10, 11] has devised a comparatively simple colorimetric method by which hemoglobin is calculated from the iron content. He found that this procedure checked very closely with the Palmer[5] method for determining hemoglobin. Lindsay, Rice and Selinger[1] report a number of analyses comparing Wong's original method[10] with the Van Slyke[12] oxygen capacity method. On the basis of seven determinations, an average variation of 2.6 per cent was obtained. They recommend the Wong method as a substitute for the Van Slyke method in standardizing hemoglobin solutions.

We felt that a longer series should be obtained before such recommendation could be made, and in obtaining such a series we determined the oxygen capacity of the blood by the manometric method of Van Slyke and Neill[9] incorporating the recommendations recently made,[13] and compared the calculated amount of hemoglobin with the hemoglobin calculated from iron by the modi-

TABLE I

SPECIMEN	VAN SLYKE PER CENT	WONG PER CENT	ACID HEMATIN PER CENT	
			VEIN	FINGER
1	12.9	13.1	12.6	
2	11.5	11.2	11.3	
3	15.3	15.7	15.1	
4	13.1	13.1	13.4	
5	13.2	13.4	13.0	
6	15.7	15.7	15.7	
7	14.9	15.1	14.5	
8	11.2	11.3	10.7	
9	14.0	14.2	13.8	13.4
10	14.3	14.0	14.5	13.8
11	11.5	11.5	11.2	11.3
12	12.7	12.6	12.5	12.2
13	12.8	12.9	12.4	12.5
14	12.8	12.8	12.3	12.7
15	14.4	14.5	14.0	14.2
16	10.5	10.3	10.2	10.2
17	12.6	12.9	13.2	12.9
18	13.2	13.6	13.3	13.2
19	11.7	11.9	11.3	11.2
20	13.5	13.7	13.8	13.5
21	12.5	12.6	12.3	12.4
22	12.1	12.3	11.6	11.7
23	12.4	12.4	11.9	12.1
24	12.3	12.0	12.8	12.5
25	13.0	13.1	13.7	13.4
26	13.0	13.3	13.0	12.6
27	13.6	13.7	13.1	13.2
28	9.8	10.0	9.7	9.5
29	9.3	9.2	9.4	9.5
30	10.0	10.1	9.8	10.0
31	12.9	13.2	12.6	12.5
32	14.7	14.5	14.1	14.4
33	12.1	12.4	12.4	12.2
34	10.7	10.9	10.5	10.5
35	13.3	13.5	13.4	13.3
36	15.7	15.9	15.2	15.4

fied method of Wong.[11] Each specimen of blood was also analyzed by the acid hematin method of Cohen and Smith and in most cases further comparison was made by an additional determination on blood from the finger (taken immediately after phlebotomy) by the acid hematin method. The standard solution was checked repeatedly by fresh standards.

The following results were obtained in a study of 36 specimens, taken from male children between the ages of six and thirteen years.

In Table I the figures represent the number of grams of hemoglobin per 100 c.c. of blood (percentage).

It is obvious from Tables I and II that there is a tendency for the acid hematin method to give slightly lower values than either the Wong or Van Slyke procedures, and to differ slightly more from the former than from the latter.

TABLE II

THE VARIATIONS BETWEEN THE DIFFERENT METHODS

SPECIMEN	PERCENTAGE OF VARIATION		
	WONG METHOD FROM VAN SLYKE METHOD	ACID HEMATIN METHOD (VEIN) FROM VAN SLYKE METHOD	ACID HEMATIN METHOD (VEIN) FROM WONG METHOD
1	1.55	2.33	3.82
2	2.61	1.74	0.89
3	2.61	1.31	3.82
4	0.00	2.29	2.29
5	1.52	1.52	2.99
6	0.00	0.00	0.00
7	1.34	2.68	3.97
8	0.89	4.46	5.31
9	1.43	1.43	2.82
10	2.10	1.40	3.57
11	0.00	2.61	2.61
12	0.79	1.57	0.79
13	0.78	3.12	3.88
14	0.00	3.91	3.91
15	0.69	2.78	3.45
16	1.90	2.86	0.97
17	2.38	4.76	2.33
18	3.03	0.76	2.21
19	1.71	3.42	5.04
20	1.48	2.22	0.73
21	0.80	1.60	2.38
22	1.65	4.13	5.69
23	0.00	4.03	4.03
24	2.44	4.07	6.67
25	0.77	5.38	4.58
26	2.31	0.00	2.26
27	0.74	3.68	4.38
28	2.04	1.02	3.00
29	1.08	1.08	2.17
30	1.00	2.00	2.97
31	2.33	2.33	4.55
32	1.36	4.08	2.76
33	2.48	2.48	0.00
34	1.87	1.87	3.67
35	1.50	0.75	0.74
36	1.27	3.18	4.40
Average	1.40	2.47	3.05
Maximum	3.03	5.38	6.67

Davis and Sheard[15] in a study of 15 specimens in which the Van Slyke method was compared with the Cohen and Smith acid hematin procedure, found an average variation of 2.5 per cent. This is of the same order of magnitude as the percentage variation obtained by us. Their maximum variation of 7.7 per cent, is, however, a little more than 2 per cent greater than ours.

TABLE III

METHOD	MEANS	DIFFERENCES	PROBABLE ERROR
Van Slyke Wong	12.55 12.65	0.10	0.2685
Van Slyke Acid Hematin (Finger)	12.55 12.35	0.20	0.2652
Van Slyke Acid Hematin (Vein)	12.55 12.45	0.10	0.2677
Wong Acid Hematin (Finger)	12.65 12.35	0.30	0.2662
Wong Acid Hematin (Vein)	12.65 12.45	0.20	0.2688

It will be seen from Table III that in every case the difference is less than three times the probable error and is therefore not significant.

TABLE IV

CORRELATION OF METHODS BY SPEARMAN RANK—ORDER FORMULA

Van Slyke and Wong	0.99
Van Slyke and Acid Hematin (Vein)	0.95
Van Slyke and Acid Hematin (Finger)	0.97
Wong and Acid Hematin (Vein)	0.95
Wong and Acid Hematin (Finger)	0.96

SUMMARY

The modified method of Wong in which hemoglobin is calculated from the iron content of the blood checked very closely with the oxygen capacity method of Van Slyke and Neill, and can therefore be used instead of the latter method in determining hemoglobin for the preparation of standard acid hematin solutions.

The acid hematin method of Cohen and Smith for the determination of hemoglobin was compared with the Wong, and Van Slyke and Neill procedures. Good checks were obtained. The simplicity and accuracy of this method warrants its use as a good clinical method. The fact that the standard acid hematin solution needed in this method can be prepared by the comparatively simple Wong procedure, makes it of general applicability.

REFERENCES

1. Lindsay, Rice, and Selinger: J. LAB. & CLIN. MED. **11**: 737, 1926.
2. Newcomer, H. S.: J. Biol. Chem. **55**: 569, 1923.
3. Cohen, B., and Smith, A. H.: J. Biol. Chem. **39**: 489, 1919.
4. Robscheit, F. S.: J. Biol. Chem. **41**: 209, 1920.
5. Palmer, W. W.: J. Biol. Chem. **33**: 119, 1918.
6. Wintrobe, and Miller: Arch. Int. Med. **43**: 96, 1929.
7. Van Slyke, and Stadie: J. Biol. Chem. **49**: 1, 1921.

8. Oser, B.: Practical Physiological Chemistry, Hawk and Bergeim, ed. 9, p. 455.
9. Van Slyke, and Neill: J. Biol. Chem. **61**: 523, 1924.
10. Wong, S. Y.: J. Biol. Chem. **55**: 421, 1923.
11. Wong, S. Y.: J. Biol. Chem. **77**: 409, 1928.
12. Van Slyke, D. D.: J. Biol. Chem. **33**: 127, 1918.
13. Van Slyke, and Sendroy: J. Biol. Chem. **73**: 121, 1927.
14. Senty, E. G.: J. Lab. & Clin. Med. **8**: 591, 1922-1923.
15. Davis and Sheard: Arch. Int. Med. **40**: 226, 1927.

A RAPID METHOD FOR DETERMINING THE TYPE OF PNEUMOCOCCUS IN SPUTUM*

By L. Rosenthal, M.D., and Bernard Sternberg, M.D., Brooklyn, N. Y.

OUR method for determining the type of pneumococcus in sputum is performed in the following way:

1. *The Homogenization of the Sputum.*—The homogenizing fluid is a combination of two components: a borax solution (Stroschein[1]) and hydrogen peroxide proposed by Sorgo,[2] and Sachs-Mücke[3] in the diagnosis of tuberculosis.

One to 3 or more c.c. of sputum are placed in a test tube. To this is added borax-boracic acid solution (borax 15 gm., boracic acid 15 gm., water 100 c.c.) a few drops at a time, until homogenization occurs. The total amount of the borax solution required varies with the character of the sputum. As a rule a quantity equal to the volume of the sputum is used. Homogenization is then completed by the addition of 3 per cent hydrogen peroxide. In most cases ¼ to ½ c.c. of the latter is sufficient; if necessary, more may be added without danger of interfering with the reaction. After the addition of the peroxide the mixture is agitated either by means of a wooden applicator stick or by shaking. In adding the various solutions, it is essential to keep the total volume as low as possible in order to procure the antigen in a concentrated form.

2. *The Preparation of the Pneumococcus Antigen.*—The tube containing the homogeneized sputum is centrifugalized for two or three minutes at high speed. The mixture then divides into three layers: a varying amount of solid material is drawn to the bottom of the tube, a foamy pellicle forms on the surface, and between these there is a zone of fluid. This fluid may be clear and transparent or it may present various degrees of opalescence. It may be aspirated by a Pasteur pipette or poured through the pellicle into a small test tube and recentrifugalized for a few seconds.

3. *The Typing.*—The following material is needed: (a) Pneumococcus antisera Type I, II, and III. (b) A wet chamber prepared by covering the bottom of a Petri dish with a piece of moist filter paper. On the filter paper two wooden applicator sticks of proper length are placed to support the glass slides. (c) Clean glass slides divided by a pencil into three sections.

A drop of antipneumococcus serum Type I is placed in the first, a drop of Type II serum in the second, and a drop of Type III serum in the third section of the slide, and a drop of the antigen is added to each. The drops of

*From the Laboratories of the United Israel Zion Hospital, Brooklyn, N. Y.

antigen and serum are carefully mixed with a glass rod. Care must be exercised to use a separate pipette and rod for each type. The slide is then placed in the wet chamber. It may be left at room temperature, but the reaction is hastened by keeping the chamber in an incubator at 37° C. The slides are examined at ten minute intervals for flocculation which may occur in a few seconds or may be delayed from ten minutes to an hour. The reaction is usually observed under the low power of the microscope but it may become so marked as to be visible to the naked eye in the proper reflection of light. The reaction that occurs with the antigen from a Type III pneumococcus sometimes varies from the usual flocculation by the appearance of mucous threads which gradually become more prominent. In some instances the antigenic fluid cannot be entirely cleared of cells and débris, but their presence does not influence the reaction and the flocculi can be easily seen between the cells. The occurrence of flocculation with one of the sera indicates the

Fig. 1.—Flocculation in homogeneized sputum mixed with corresponding antiserum (wet preparation: Zeiss, Objective A. Ocular 10x).

corresponding type. The absence of flocculation with all the three may either indicate Type IV or it may be due to the insufficiency of antigen I, II, or III in the sputum. In nearly all of our cases the absence of the reaction was due to the fact that the sputum contained Type IV organisms.

In order to establish the nature of the flocculation, the following examination was done:

A mixture of a drop of homogeneized pneumococcus sputum and a drop of the corresponding serum was allowed to dry on a slide. The film was fixed with methyl alcohol and then stained with methylene blue and examined microscopically under the high power. In most cases we found precipitated granular flocculi (Fig. 1) but at times we could also see masses of agglutinated pneumococci (Fig. 2). The reaction must therefore be regarded as a combined agglutination-precipitation test.

We typed by our method 81 sputa from patients with lobar pneumonia which we obtained during the months from October, 1928 to February, 1929

from the various hospitals (United Israel Zion, Beth Moses, Greenpoint, Crown Heights). Every specimen was checked by the mouse inoculation method.

Seventy-four sputa showed identical results in both methods, namely: 15 cases Type I, 16 cases Type II, 12 cases Type III, 27 cases Type IV, 4 cases Types II and III.

Four sputa gave flocculation with all three undiluted sera. In these cases we repeated the test using diluted sera in order to delineate the reaction more clearly. Dilution was performed as follows:

Type I serum diluted 1:10 by addition of one drop of serum to 9 drops of saline;

Type II serum diluted 1:10 in the same manner;

Type III serum diluted 1:5 by adding 2 drops of serum to 8 drops of saline.

All four sputa when tested with the diluted sera, reacted according to their respective types, viz.: 2 cases Type I, 2 cases Type IV.

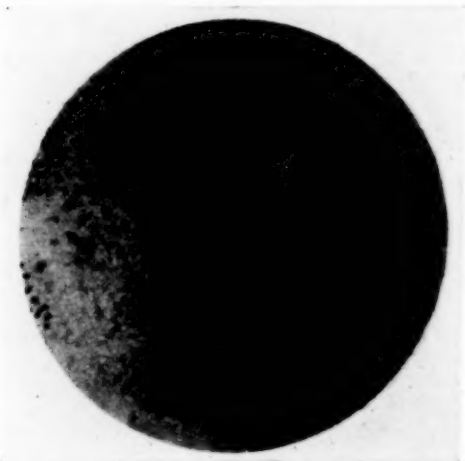

Fig. 2.—Agglutinated pneumococci in homogeneized sputum mixed with corresponding antiserum (Zeiss, Oil Immers., Ocular 10x).

Only 3 sputa showed a discrepancy between the mouse inoculation method and our method, viz.:

Mouse method	*Homogeneization method*
Two cases Type I.	No flocculation with any of
One case Type II.	the sera.

The discrepancy may be explained by the assumption that these sputa did not contain a sufficient amount of antigen to be detected by our test.

We also typed by our homogeneization micromethod pneumococcus pus from nine cases of empyema with the following results: 4 cases Type I, 2 cases Type II, 1 case Type III, 2 cases Type IV. These findings were confirmed by typing the cultures obtained from the pus. It is necessary to add that results were positive in 5 cases not only by the homogeneization procedure but also by direct typing of the liquid part of the untreated pus. In the other four cases homogeneization of the pus was required to establish the type.

We also applied our method to the residua of 10 sputa which are discarded as useless after extraction of the antigen by the method of Krumwiede and Valentine[4] and Oliver.[5] We found that these residua still contained sufficient antigen which could be demonstrated by flocculation with the corresponding serum.

CONCLUSIONS

The homogeneization micromethod for the determination of the type of pneumococcus in sputum, pus, etc., fully described above, has the following advantages:

1. It allows the direct typing of the material without the use of animals or culturing methods.
2. It makes possible the determination of the type in a short time.
3. It requires relatively small quantities of material.
4. It permits more complete utilization of the pneumococcus antigen contained in the sputum.

The reliability of our procedure is vouchsafed by the nearly full coincidence of its findings with the results of the mouse inoculation method.

REFERENCES

1. Stroschein: Mitt. a. v. Brehmer'schen Heilanstalten **1**: 289, 1889.
2. Sorgo: Wien. klin. Wchnschr. **26**: 1447, 1903.
3. Saehs-Mücke: München. med. Wchnschr. **53**: 1660, 1906.
4. Krumwiede and Valentine: J. A. M. A. **70**: 513, 1918.
5. Oliver: J. Infect. Dis. **27**: 310, 1920; **29**: 518, 1921.

A METHOD OF USING WRIGHT'S STAIN IN STAINING JARS*

By Roy F. Feemster, M.D., D.P.H., New Orleans, La.

THERE has been a long-felt want of a method by which blood smears and other preparations might be successfully stained in quantity by Wright's Stain, without handling each slide individually as has been necessary heretofore. Many of us have attempted to devise a method by which this might be done, but preparations obtained were never satisfactory.

Since describing a modified procedure of applying Wright's Stain[1] and making a study of the variable factors in the use of this stain,[2] we have been able to work out a procedure by which this modified Wright's Stain can be used in staining jars.

The jars used in this procedure are set up as follows:

1. Solution I[1]
2. Buffered Water[1]
3. Solution II[1]
4. Solution II
5. Buffered Water
6. Buffered Water

In order to stain several slides at once some kind of holder is necessary. The Miller slide holder (Will Corporation, Catalog No. 15672), used in many

*From the Department of Bacteriology and Pathology of Tulane University.
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laboratories, is very satisfactory. It consists simply of a number of hard rubber leaves between which the slides are placed, the slides being held securely when a thumb screw is tightened. The holder rests on the top of the staining jar, the slides hanging down into the solutions.

The solutions should come to about one-half an inch of the top of the staining jars. An ordinary straight-sided tumbler makes a satisfactory staining jar. It should be small so that the quantities of solution will not have to be too great.

The staining procedure is as follows:

1. Place slides in Solution I for one minute.

2. Remove, drain off as much stain as possible; stand on end on a blotter or filter paper and *allow stain remaining to turn red.*

3. Place in buffered water for three minutes or longer.

4. Remove from water, drain or sling off as much as possible, and dip rapidly two or three times in Solution II (Jar 3).

5. Dip three or four times in Solution II (Jar 4); or allow to stand in this jar for fifteen or twenty seconds.

6. Wash in buffered water (Jar 5).

7. Wash in buffered water (Jar 6).

8. Stand on end until dry. A staining rack which is very useful here has been described.[3]

The buffered water should be changed frequently, as it soon becomes colored with methylene blue. Solutions I and II should be kept covered tightly when not in use. Petri dishes make fairly satisfactory covers for tumblers used as staining jars. When stains are not used constantly, it is much better to pour Solutions I and II back into stoppered bottles. Solution II from Jar 3 should not be mixed with that from Jar 4. That in Jar 3 should be discarded from time to time, the solution from Jar 4 being poured into Jar 3 and new solution placed in Jar 4.

When the alcohol has evaporated from Solution I the volume may be brought back to the original level by adding more *methyl alcohol.* Solution II, however, *cannot be made up to volume by adding ethyl alcohol* as the solution will not again become saturated for a considerable length of time after the addition of the alcohol. For this reason the level should always be brought back by *adding more of Solution II.*

Single slides are also easily stained by this method, of course, it being simpler in some ways than staining by pouring solutions on the slides. There is an added advantage of having even cleaner preparations.

REFERENCES

1. Feemster, Roy F., and Feemster, Olive S.: A Blood Stain Giving More Constant Results, J. Lab. & Clin. Med. **13**: 1139, 1928.
2. Feemster, Roy F.: A Study of the Variable Factors in the Use of the Wright's Stain, Ann. Int. Med. **2**: 289, 1928.
3. Feemster, Roy F.: A Useful, Simply Constructed Staining Rack, J. A. M. A. **88**: 2035, 1927.

THE APPLICATION OF THE TWORT-D'HERELLE PHENOMENON IN THE DIAGNOSIS OF SHIGA DYSENTERY*

By Joseph C. Willett, D.V.M., St. Louis, Mo.

THE bacteriophage phenomenon is being intensely studied in its application to the treatment of infectious diseases. In certain infections phage products have proved their usefulness as therapeutic agents.[1] The application of this principle in the Public Health Laboratory has stimulated intense work in the solving of many bacteriologic problems. It is the object of this article to discuss the use of this principle as a practical laboratory procedure in differentiating between strains of dysentery bacilli and organisms of the typhoid, paratyphoid group.

A discussion of the many difficulties encountered in differentiating the pathogenic intestinal organisms is beyond the scope of this article. It is well known, however, that occasional freshly isolated field strains of the typhoid, paratyphoid, and dysentery bacilli group do not readily yield to the various laboratory procedures of differentiation commonly used. The use of the phage principle in these cases promises to expedite diagnosis and also serve as an additional check on other differential procedures.

The phage used in this study was isolated from the stool of a typhoid termination case. Its activity was tested against a variety of organisms including: dysentery bacilli; Shiga, Flexner and Hiss. Y.; various stock and field cultures of B. typhosus, B. paratyphosus A and B, and B. coli. It proved active against Shiga, Flexner and Hiss. Y. and inactive against other organisms. However, it was decidedly more active against the Shiga strain.

Several surveys were made during the past summer in St. Louis in search of typhoid carriers. In the course of the work we encountered four patients from whom organisms, giving certain cultural characteristics of dysentery bacilli, were isolated. These freshly isolated strains were atypical in their biologic behavior, so the phage principle was used in their identification. Broth transplants of these field cultures were inoculated with anti-Shiga phage and after twenty-four hours' incubation complete lysis was observed. This work was controlled with stock strains of Shiga and stock and field strains of B. typhosus and by omitting phage from transplants of the field culture. With further study these field cultures gave typical sugar reactions and were agglutinated by anti-Shiga serum. Table I shows typical results obtained with the various test cultures after twenty-four hours' incubation.

The clinical histories in these cases did not indicate clearly whether they were chronic convalescent carriers resulting from mild attacks of dysentery or true carriers resulting from contact with active cases. None of these cases

*From the St. Louis Health Division Laboratories.
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TABLE I

ACTION OF ANTI-SHIGA PHAGE ON BROTH CULTURES*

	UNIDENTIFIED CULTURE	STOCK SHIGA	STOCK HISS. Y	STOCK FLEXNER	STOCK TYPHOID	FIELD TYPHOID
Inoculated with phage	++++	++++	++	+	−	−
Not inoculated with phage	−	−	−	−	−	−

*Four plus (++++) indicates complete lysis of culture and negative (−) absence of lysis.

showed clinical evidence of the disease at the time the specimens were taken. In this connection, Nichols[2] suggests that Shiga infections more frequently indicate chronic bacillary dysentery.

Although numerous studies of the phage principle are based upon its action against Shiga's dysentery bacilli, we find no mention of the practical use of this principle in connection with differentiation between Shiga and organisms of the typhoid and paratyphoid group for the purpose of diagnosis. It is evident, however, that it can be used in this connection only when careful consideration is given to the complexity of this principle. Extensive literature on this subject is adequately reviewed by Hadley[3] so will not be discussed here.

While striving to attain the objective indicated by Bronfenbrenner[4] who says "the hope of utilizing bacteriophage as an agent for prevention and therapy of infection lies in finding the means of removing the obstacles to its activity in its natural environment and allowing it to act freely as it does in a test tube," it is well to study the possibilities of the practical use of this principle in differentiating pathogenic bacteria for the purpose of diagnosis and in the positive identification of organisms of sanitary significance in food and water.

REFERENCES

1. D'Herelle, F.: The Bacteriophage and Its Behavior, 1926, Baltimore, Williams and Wilkins Company.
2. Nichols, H. J.: Carriers in Infectious Diseases, 1922, Baltimore, Williams and Wilkins Company.
3. Hadley, P.: The Twort-d'Herelle Phenomena, J. Infect. Dis. **42**: 263, 1928.
4. Jordan, E. O., and Falk, I.: The Newer Knowledge of Bacteriology and Immunology, 1928, The University of Chicago Press.

A RAPID BLOOD GROUPING METHOD*

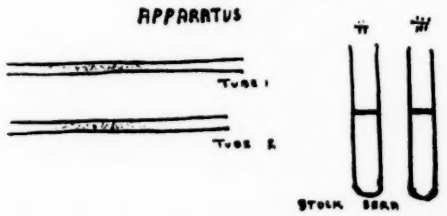
By Nathan Grosof, M.D., Brooklyn, N. Y.

APPARATUS

CAPILLARY tubes, medium size, 6 inches long. Capillary tubes, medium size, 5 inches long. Stock sera, Group II and Group III. Suspensions of recipient's and donor's red cells. Sera of donor and recipient, collected in Wright's capsule tubes.

PROCEDURE

The shorter capillary tube is dipped into the Group II stock serum which is drawn by capillary attraction to one-half the distance. Immediately the tube is placed within the recipient's cell suspension, care being taken not to allow



air bubbles to enter between serum and cell suspension. The capillary tube is then held at either end by the thumb and index finger and inverted, allowing the cells to gravitate into the serum. This procedure is repeated with the longer capillary tube, using the type three stock serum.

Both tubes are placed under the low power lens and by proper focus one can readily note within five minutes the final result. Agglutination appears as fine cayenne pepper clumps. The eyepiece itself may be used as a direct focus upon the capillary tube.

Direct matching may be carried out in a similar manner, dipping the capillary tubes directly into the Wright capsules.

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*From the Department of Pathology of the Brownsville and East New York Hospital.
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ELECTROCUTION IN SACRIFICING LABORATORY ANIMALS*

By Leo S. Hrdina, Chicago, Ill.

ELECTROCUTION deserves wider use in sacrificing laboratory animals than it has received. No means for ending life is as humane. Consciousness is instantly interrupted. At application of the current the animal stiffens and all functions cease without struggling or other agonal symptoms. Thus, no lesions are produced or chemicals introduced that may interfere with experimental findings. The method is extremely simple and applicable wherever there is 110 V. alternating current, for higher voltages are entirely superfluous. (With animals larger than dogs I have had no experience.) Expense and time are minimized, and these may be factors of significance where numbers of animals are dealt with.

APPARATUS

One 10-foot length of double-ply electric light cord, fitted at one end with a standard "plug-in" fixture and at the other end with two metal spring clips as used for storage battery contacts. At the end with clips the cord is untwisted for 2 or 3 feet, to allow the clips to be separated.

METHOD

The hair on the top of the head and on the side of one thigh is wet to the skin with water, and the clips attached to the skin. If wetting is thorough, the contact is sufficient and other preparation unnecessary. The plug is then inserted in an electric outlet and left in circuit about two minutes. If on release heart action resumes, another application is made.

*From Department of Surgery, University of Chicago.
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DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

LEAD POISONING: A Critical Survey of the Methods for the Determination of Lead in Biological Material, Tannahill, R. W. Med. J. Australia 1: No. 16, 19, 1929.

The following method is recommended as sensitive to 1 in 10,000,000.

DETERMINATION OF LEAD IN URINE

Evaporation of the urine: Place 500 c.c. of the sample in a 7 inch porcelain basin, add 50 c.c. of nitric acid and evaporate on the hot plate, gradually adding another 500 c.c. of the sample, making one liter in all. Continue the evaporation until about 30 c.c. are left.

Destruction of organic matter: Transfer to a silica dish (about 9 cm., 3½ inches, in diameter) cleaning out the porcelain basin with 10 c.c. of nitric acid and finally washing it with distilled water. Allow the contents of the dish to evaporate slowly on the hot plate to avoid loss by spraying. Continue the evaporation to dryness, being careful to avoid overheating which causes the mass to fume strongly and possibly to deflagrate. When dry, place the dish in a small muffle, preferably electrically heated, and heat until fuming ceases and the mass becomes charred, proceeding very cautiously to prevent deflagration, especially at first. Gradually increase the temperature by pushing the dish farther into the muffle until the carbon burns off quietly and the mass becomes white. Heat to between 450° and 500° C. until red fumes cease to be given off, usually in about twenty minutes. If the nitrates are not completely decomposed at this stage, the analysis will have to be rejected.

Separation of second-group metals: Cool, add hot water until the dish is about two-thirds full; heat to boiling on the hot plate, break up lumps with a glass rod and carefully add hydrochloric acid until the solution is clear and either neutral or very slightly acid. Then add exactly 2 cm. of six times normal hydrochloric acid in excess. Special attention must be paid to the acidity of the solution at this stage. The white residue after ignition is usually alkaline and must be carefully neutralized; then the required excess of acid is added. Filter off any small residue of silica through a 9 cm. filter paper, wash well with hot water, catching the filtrate in a 300 c.c. Erlenmeyer flask. Cool and make up the bulk to 250 c.c. Fit the flask with a rubber stopper with two holes, through one of which a straight tube passes nearly to the bottom of the flask, the end of this tube being drawn out to a fine jet. Through the other hole is passed another glass tube bent at right angles, going through the stopper for a length of about 12 mm. (half an inch). Connect up with the hydrogen sulphide apparatus and bubble a slow stream of the gas through the cold solution for one hour. Allow to stand overnight, when any precipitate will settle out. Filter through a 9 cm. filter paper, wash out the flask three times with a small quantity of cold hydrogen sulphide water and then wash the filter paper three times with the same liquid.

Separation of the lead as sulphate: Dissolve the precipitate of sulphides by dropping 2 c.c. of hot 1:1 nitric acid from a pipette around the filter paper, followed by another 2 c.c. of the same, catching the solution in the original Erlenmeyer flask. Wash six times with hot distilled water. Warm the solution in the flask and evaporate to about 20 c.c., then transfer to 50 c.c. cylindrical beaker (nonalkali glass), washing out the flask 3 times with warm water. Evaporate quietly to about 5 c.c. on the hot plate, then add 1 c.c. of sulphuric acid and heat until strong fumes arise. Cool and add 20 c.c. of cold water, then 10 c.c. of alcohol; mix well and allow to stand overnight. Filter through a 9 cm. filter paper, washing out the beaker with a solution of 65 volumes water, 32 volumes of absolute alcohol and 3 volumes of sulphuric acid. Then wash the filter paper 3 times with the same solution.

Colorimetric determination of the lead: Dissolve any precipitate off the filter paper by dropping 5 c.c. of hot (1:2) ammonium acetate solution from a pipette around the filter paper, followed by another 5 c.c. of the same reagent, catching the solution in the original 50 c.c. beaker. Wash 6 times with hot water. Cool and Nesslerize, using a standard solution of lead acetate of 0.0001 gm. of lead in 1 c.c.

The Nesslerizing is carried out in the following manner: Transfer the assay solution to a 50 c.c. Nessler tube, add 1 c.c. of a 10 per cent potassium cyanide solution, 1 c.c. of ammonium hydroxide and 6 drops of freshly prepared ammonium sulphide. Make up to the 50 c.c. mark and mix well. This assay solution must be quite colorless. Add to it the standard lead acetate solution, stirring after each addition until the color matches that of the assay, when both tubes are placed in a colorimeter. It is advisable to arrange the strength of the assay solution so that not more than 8 c.c. of the standard lead solution will be required for Nesslerizing. If much lead sulphate precipitate is noted after the addition of water and alcohol, the solution in ammonium acetate must be made up to a definite volume and an aliquot part taken for Nesslerizing, so that not more than 8 c.c. of standard solution will be used in the comparison. In this case use the same proportion of (1:2) ammonium acetate for making up the comparison solution as is contained in the aliquot part of the assay solution. It is desirable in all cases to regard the first comparison as a trial and to make up another standard in which the required lead acetate solution is added before the ammonium sulphide and along with the ammonium acetate, potassium cyanide, ammonia and water to make up a total of 50 c.c. The solution is mixed well and 0.3 c.c. of ammonium sulphide added and after further mixing compared with the assay. Slight additions may then be made to either assay or standard in order to match them and the necessary corrections made.

THE CHROMATE METHOD FOR THE DETERMINATION OF LEAD

Ashing: Before analysis, tissues and feces must be freed from water by baking. This may be done very rapidly by heating the material in porcelain dishes on a hot plate until it starts to char. Transfer to the muffle furnace and ash to a dull red heat. Fecal matter usually ashes readily, but the tissues form a residue which must be repeatedly extracted before the entire char is consumed. Usually most material requires reashing as a certain quantity of inorganic salts becomes fused and prevents complete oxidation. After the first ashing, the material should be cooled and extracted with dilute hydrochloric acid and hot water. It is essential that at this stage all the ash be dissolved, for frequently lead phosphate is present as an insoluble residue that might be mistaken for silica. If this residue is insoluble in hydrochloric acid, it should be treated with a mixture of hydrochloric acid and tartaric acids (which dissolves lead phosphate) until the ash is quantitatively dissolved.

In analyzing urine for lead, the procedure has been to evaporate to dryness, char and ash the residue. Since urine residues are difficult to ash, because of the large quantity of inorganic salts present, repeated extraction and ashing is necessary if all the salts are to be dissolved. This makes the process burdensome. The following new method in which evaporation is avoided by precipitating lead directly from the urine, has been devised.

Entrainment: Ammonium hydroxide is added to urine until it is strongly ammoniacal. The mixture is allowed to stand from one to twenty-four hours. In this reaction the earthy phosphates are precipitated and lead phosphate is carried down quantitatively by entrainment. The gelatinous mass of phosphates settles into a compact mass from which the clear lead-free liquor can be decanted and the remainder filtered by suction on a Buchner funnel. The filter paper containing the precipitate, ashes readily in a few minutes and the quantity of lead may be determined by the chromate method as follows:

Precipitation: The strongly acid solution should be neutralized with sodium hydroxide. Hydrochloric acid should be added until the solution is just acid to methyl orange. Saturate the cold solution with hydrogen sulphide; if sulphides precipitate to any great extent during the process, they may be filtered at once, but if no precipitate appears, the solution saturated with hydrogen sulphide should be allowed to stand overnight before filtration. Immediately after filtration the precipitate should be washed as lead sulphide oxidizes rapidly when in

contact with air. Solution of the washed precipitate in nitric acid, boiling to expel hydrogen sulphide, cooling and finally neutralizing with sodium hydroxide, as indicated by phenolphthalein, are then necessary. After reacidification with acetic acid, two or three drops of a saturated solution of potassium chromate should be added. To hasten the reaction the solution should be boiled for a few minutes. If no turbidity is present, the solution should stand overnight before filtration to allow separation of the extremely small amount of lead it may contain. After filtering, all trace of soluble chromate should be washed from the filter paper.

Titration: The chromate is dissolved in dilute hydrochloric acid, an excess of potassium iodide added at once and the free iodine titrated with 0.005 normal thiosulphate solution, starch being used as an indicator.

ACETONE IN BLOOD AND URINE: A Method for the Detection of Acetone, Wallhauser, A. J. A. M. A. **91:** 31, 1928.

Reagent (Scott-Wilson):

"Ten grams of mercuric cyanide is dissolved in 600 c.c. of water. Add a cooled solution of 180 gm. of sodium hydroxide in 600 c.c. of water. Transfer this mixture to a heavy glass jar and add 2.9 gm. of silver nitrate dissolved in 400 c.c. of water. This must be added in a fine slow stream with vigorous stirring constantly. The silver dissolves completely, giving a clear solution. If it is turbid it may be set aside, and the clear supernatant fluid removed by a siphon after three or four days. This solution will keep for six months, after which a new solution must be made. Keep in a tightly stoppered dark bottle." The stopper of the container may conveniently carry a pipette graduated in twentieths of a cubic centimeter. A rubber bulb should not be used on the pipette.

Method: The urine or blood is placed in a suitable container. The original container used in most hospitals for urine specimens is generally employed, no attempt being made to measure the volume. One drop of Scott-Wilson reagent is placed on a microscopic slide, which is then inverted to form a hanging drop. This is placed over the mouth of the container, care being taken that the reagent does not come in contact with the neck of the bottle, in order that precipitation may not occur through direct contact with the urine. Any time of exposure may be made, but an exposure of two minutes has been established, as this period has been found to give the best quantitative results. After the time period has elapsed the hanging drop is inspected for the macroscopic appearance of a fine white clouding or precipitate. If there is such a precipitate or clouding, the test is positive for acetone. If the reagent remains unchanged, acetone is negative.

ACHYLIA GASTRICA: The Excretion of Neutral Red in the Stomach, Winkelstein, A. and Marcus, J. M. J. A. M. A. **92:** No. 15, 1238, 1929.

The following modified technic is described:

The patients presented themselves with fasting stomachs, the Rehfuss tube was passed, and 40 mg. of neutral red dissolved in a few cubic centimeters of sterile distilled water was injected intramuscularly (gluteal or deltoid). The patients were then given, at once, 250 c.c. of strained oatmeal gruel. A sample was aspirated every fifteen minutes for two hours. This procedure was carried out in 60 cases of achylia gastrica. All of these patients had had an Ewald and at least one fractional test meal and often two or three with bouillon, alcohol or oatmeal gruel. In 15 cases, histamine (Imido-Roche, 7½ minims, 0.45 c.c., subcutaneously) was administered in addition to the neutral red and the test meal. Neither the dye nor free hydrochloric acid appeared in the gastric contents. With reference to certain criticisms, one should state that bile, stained reddish brown by the dye, can readily be excluded by the practiced eye as a source of error. A consideration of the results of this study shows that, irrespective of the disease present, the dyestuff is not excreted for two hours when a true achlorhydria is present. In the group are included 6 cases of pernicious anemia, 10 of gastric carcinoma, 3 of cholelithiasis, 17 patients with subtotal gastrectomy for ulcer, and 2 proved cases of gastric syphilis. It is obvious that true achylia gastrica,

or, better expressed, true achlorhydria, occurs in a variety of conditions and that the failure of the neutral red to appear is linked up with the absence of the acid and is not determined by the disease present.

The following conclusions are advanced:

1. Neutral red is excreted into the stomach whenever free hydrochloric acid is secreted.
2. Neutral red is invariably excreted in the false achlorhydrias.
3. It is not excreted in true achylia gastrica, whatever the cause of the achylia may be.
4. Neutral red gives as much information as histamine and is preferable to the latter for routine use.
5. Neutral red is helpful in the study of the normal and pathologic physiology of gastric secretion and in the differential diagnosis of the diseases producing false and true achylia gastrica.
6. The use of neutral red in every case in which achylia gastrica is suspected is advocated.

GONORRHEA: The Oxidase Reaction in the Laboratory Diagnosis of, Price, I. N. O. British M. J., 199, February 2, 1929.

After microscopic examination of suspicious forty-eight hour colonies, if the organisms resemble gonococci morphologically the surface of the medium is gently washed with about 0.5 c.c. of 1 per cent solution of dimethyl-paraphenylene-diamine hydrochloride and examined after one, three, five, ten, fifteen, twenty, and thirty minutes for any color change in the colony under observation.

A positive reaction is shown by the appearance (in gonococcus) of a gradually deepening pink color, changed to red, then to reddish-purple, and finally in thirty minutes to jet black.

The same reaction is given by M. catarrhalis but appears more rapidly.

GONOCOCCUS: Successful Cultivation on Blood Agar Plates, Herrold, R. D. J. Infect. Dis. **42**: No. 1, 79, 1928.

Formula:

10 gm. peptone Witte
7.5 to 10 gm. bactoagar
1 gm. dibasic potassium phosphate
50 gm. bactobeef dehydrated

The bactobeef is added to one liter of water and after standing overnight the juice is squeezed out and made up to one liter with distilled water. The reaction is adjusted to P_H 7.4 to 7.6.

To the melted agar at 65° C. add 10 to 15 per cent of defibrinated sheep blood or whole human blood, cool to 45° C. and pour as plates or slants.

In the author's experience this medium is very successful for primary cultures.

FLAGELLA: A Method of Staining Bacterial Flagella, Craigie, J. Brit. J. Exper. Path. **9**: No. 2, 55, 1928.

This is essentially a modification of Zettnow's method.

The usual precautions as to clean glassware are obligatory.

Use twenty-four-hour cultures on agar with plenty of water of condensation and pipette off the growth in the condensation water, which is at once dropped into "fixative solution." The fixative solution consists of saline containing 2 per cent of formalin. Formolized phosphate buffer solution of P_{H_7} may be used instead of saline. Leave in fixative solution for one hour or, better, overnight, before attempting to stain. These formolized suspensions keep for months at room temperature.

Preparation of Films: In a clean tube put distilled water and add thereto the formolized suspension until faint opacity appears. Spread gently a small loopful of this on

a slide cleaned as above. Dry at 37° C., the slide being inclined so that varying degrees of thickness of suspension are obtained .Then heat at 90° to 100° C. for five minutes or longer. (This can be done conveniently on the lid of a small steam sterilizer such as is used for sterilizing pipettes or syringes.)

Place in jar of distilled water for five minutes and wash with distilled water. Dry and heat again at 90° to 100° C., when preparations may be mordanted.

Mordanting: Dissolve 10 gm. of tannic acid (this must be "light and pure," not of commercial quality) in 200 c.c. of distilled water, heat to about 60° C., and slowly add, with frequent agitation, 30 c.c. of 5 per cent aqueous solution of tartar emetic. A crystal of thymol is necessary if the mordant is to be stored.

The slide is flooded with mordant and heated for five to ten minutes at 90° to 100° C. When doing this the slides should be under constant observation, and when any tendency to drying of the mordant is observed at the edges of the slides more mordant should be run on and guided along the dry edge with a small glass rod. Remove slide and flood off mordant under tap. Any dry mordant at the edges of the slides, which is easily seen, as it becomes white on cooling, must be removed with a clean cloth. Wash in distilled water.

Silvering: Dissolve 1 gm. silver sulphate (B. D. H. brand used) in 250 c.c. of distilled water and store in light-tight bottle.

To 20 c.c. of this silver sulphate solution add mono-ethylamine solution (33 per cent B. D. H.) until the resulting opacity just clears up. More silver may be added if the ethylamine has been added in excess. About 4 to 5 drops of ethylamine are required for 20 c.c. of silver solution. Flood slides with "silver ethylamine" mixture thus prepared, and warm over tip of luminous flame until preparation just begins to steam. Allow to continue steaming, but do not overheat, until the preparation turns brown or black according to degree of reduction. It is emphasized that to obtain the best preparations heating should not be continued after the preparation has turned brown. If a black metallic cloud appears in the solution, wash off and replace with fresh "silver ethylamine." When sufficiently reduced flood off under tap, care being so taken as to flood the slide that no metallic film settles on the preparation. Wash in distilled water. (If desired one can mount the preparation after drying, but such preparations fade rapidly, especially if the silver has not been completely reduced, and it has already been noted that complete reduction does not give optimum preparations.)

Gold "toning": Prepare toning solution by adding 20 drops of 1 per cent solution of gold chloride to 20 c.c. of distilled water. The gold solution was made from ordinary photographic gold chloride. Immerse slides in this weak solution of gold chloride and expose to daylight for thirty minutes or longer. Wash and dry.

When greater density and contrast is desired the following alternative to gold "toning" may be adopted with advantage.

Immerse slide for five minutes or longer in a 0.1 per cent solution of uranium chloride. Wash and apply the following developer for one to two minutes:

Pyrogallic acid (Merck), 0.5 per cent aqueous solution, 10 c.c.

Liquor ammoniae fort., 3 to 5 drops.

Mix and use immediately.

Wash slide and dry.

This developer may be applied also to the gold preparation.

Mounting preparations: Preparations are mounted in balsam without solvent, the balsam being rendered fluid by heat. This method gives very delicate permanent preparations.

OLIGODENDROGLIA: Method of Staining Oligodendroglia and Microglia, Penfield, W. Am. J. Path. 4: No. 2, 153, 1928.

1. Harden: Tissue in 10 per cent formalin (or formalin-ammonium-bromide) for an indefinite period. About a week in formalin gives excellent results.

2. Section: Cut sections at 20 microns on the freezing microtome and receive them in 1 per cent formalin or distilled water. Through the succeeding steps the sections should be handled by a glass rod shaped like a hockey stick.

3. Deformalinize: Place sections in dish of distilled water to which 10 to 15 drops of strong ammonia have been added and cover so as to prevent escape of ammonia. Leave in this solution overnight to remove formalin.

4. Bromuate: Transfer sections directly to Globus' hydrobromic acid in 5 per cent solution (5 c.c. of 40 per cent hydrobromic acid plus 95 c.c. distilled water). Place in incubator at 38° C. for one hour.

5. Wash: Pass through three changes of water (a, b, c).

6. Mordant: Place sections in 5 per cent solution of sodium carbonate for one hour. (Sections may remain here five to six hours without ill effect.)

7. Impregnate: Pass sections with or without washing direct to Del Rio-Hortega's silver carbonate, weak solution,* and leave them here three to five minutes. Sometimes they may be left until they begin to turn a yellowish gray. Then transfer them to the reducer. Control the duration in silver solution by taking out a section at intervals of one to two minutes and examining under the microscope. The sections should turn a smooth gray color in the following reducer.

8. Reduce: Plunge into 1 per cent formalin and agitate.

9. Wash: Distilled water.

10. Tone: Leave in gold chloride (1-500) at room temperature until all yellow tint disappears and the sections are a smooth bluish gray.

11. Fix: Hyposulphite of soda (5 per cent photographic "hypo").

12. Wash: Distilled water.

13. Dehydration may be done conveniently after Del Rio-Hortega's custom as follows: Float sections on the slide and flatten out with needle. Wash with two to four changes of 95 per cent alcohol from a drop bottle. Follow this with a few drops of carbol-xylol-creosote (proportion of 1-15). When clear, drain slide and blot immediately with two thicknesses of fine filter paper. Mount in Canada balsam.

ACIDOPHILUS MILK: Preparation of, Rice, F. E. Am. J. Pub. Health **9**: 1105, 1928.

Carefully clean a quart thermos bottle by allowing it to stand overnight full of water containing some washing powder or a little household ammonia; then discard it. Place the cork, a can opener and thermometer in a pan and pour boiling water over them. Wipe the top of a one-pound can of evaporated milk free from dust and pour boiling water over it. Open the can and pour contents into the pan that has been scalded. Fill the can with boiling water and pour into the evaporated milk. Immerse the pan in cool water and stir the mixture with the thermometer until the temperature comes down to about 105° F. Add 2 or 3 ounces of commercial B. acidophilus culture, mix, and transfer to the thermos bottle. (The temperature should now be between 100° and 102° F.) Cork and let stand for twenty-four hours, or until the milk has acquired a pleasantly sour taste. When this is attained, transfer to a clean milk bottle and place in the refrigerator.

Succeeding cultures of acidophilus are prepared by using about a teacupful (6 ounces) of milk culture previously made to inoculate the diluted evaporated milk for the next run. Proceeding in such a manner it will be found that acid is produced at a more rapid rate than when the first quart was prepared using the commercial broth culture as a starter. Thirteen to seventeen hours are now quite sufficient. If fermentation is allowed to proceed for a longer time so much acid is developed that the taste becomes unpleasantly sour.

After a little experience one may stop the action of the bacteria at any desired degree of sourness. This is effected by merely transferring the milk to a clean glass bottle and placing in the refrigerator. It is perfectly safe to keep the culture at room temperature, but,

*Silver nitrate (Merck), 10 per cent solution ..... 5 c.c.
Sodium carbonate (pure), 5 per cent solution ..... 20 c.c.
Ammonium hydroxide (sufficient to dissolve the precipitate).
Distilled water, ad. ..... 75 c.c.

The ammonium hydroxide in strong, fresh solution should be added drop by drop until the precipitate is just dissolved, stirring the solution all the while. It is important not to add too much ammonia. A fine black sediment may remain behind which does not resemble the more voluminous precipitate of silver carbonate. This fine sediment should be filtered off. The solution may then be preserved in a dark bottle for long periods.

as has already been mentioned, a considerable increase in acidity may be expected. This is of little consequence, however, if the product is consumed within twenty-four hours. On the other hand, if the milk is kept in a refrigerator it should be consumed within forty-eight hours for the reason that the organisms rapidly die out at such low temperatures.

SPIROCHETES: Some New Spirochete Stains, Gulstein, M. and Dhar, D. R. Dermat. Wchnschr. **86:** 45, 1928.

1. Ferri-(Chrom)-sulphate-tannin-methyl violet stain: A concentrated solution of ferri sulphate, chrom sulphate, or chrom alum; 30 per cent tannin solution; 1 per cent watery methyl violet. Treponema is suspended in two or three loops of first solution and rubbed into it on the microscopic slide. A few minutes later specimens are prepared by wiping the mixture on slides. They are dried, and fixed by heating. Then they are soaked in distilled water, and tannin solution poured over them. It is left for five minutes or longer, rinsed, and the methyl violet left on for three or five minutes.

2. Carbolfuchsin-tannin-potassium antimonyl tartrate-methylene blue stain: Aside from Ziehl's solution, 5 per cent tannin, 10 per cent solution of potassium antimonyl tartrate, and 1 per cent watery solution of methylene blue. After the smears have been fixed with alcohol or by heat the oral spirochetes are stained with Ziehl's solution, boiling for two to five minutes, rinsed and treated with tannin solution. After second rinsing exposed to the potassium solution for five minutes, renewed rinsing and exposed for from one to three minutes to methylene blue. If methylene stain is continued too long spirochetes also take the blue color. Treponema stain held light red, while bacteria and cocci have a red body and blue ectoplasm.

3. Supravital stain with Victoria dyes: A loop of buccal spirochetes is rubbed on a slide with a drop of physiologic saline. Then two or three loops of a 2 to 5 per 1,000 solution of Victoria blue are added, well mixed, and a cover glass is applied.

NEUROGLIA: Staining Fibrillary Neuroglia in Formalin Fixed Material, Davidoff, L. M. Am. J. Path. **4:** No. 5, 493, 1928.

1. Blocks of tissue 2 or 3 mm. thick are cut from the formalin-fixed brain, cord, or tumor, and placed in a dish filled with about 100 c.c. of distilled water containing 30 to 40 drops of strong ammonia water. This is kept air-tight in an oven at 30° C. for four days.
2. The blocks are washed for twelve to twenty-four hours in running water.
3. They are then fixed in Zenker's solution for twenty-four hours.
4. Embed, cut, and stain.

The Stain:

1. Mix ethyl violet 1.0 gm.
   orange-G 0.5
   } accurately weighed
2. Add 100 c.c. distilled water and stir thoroughly.
3. Place in or on a warm oven (37° C.) for twelve to twenty-four hours to precipitate.
4. Decant supernatant fluid and wash precipitate several times with distilled water.
5. Place in oven to dry.
6. Make a saturated solution of dried precipitate in absolute alcohol.

This solution, if well stoppered, will keep indefinitely.

For staining, use one part stock solution to three parts of 20 per cent alcohol.

REVIEWS

Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.

*Diseases of the Stomach**

IN THE production of this volume Dr. Rehfuss has made a great contribution to medicine. It contains a veritable mine of valuable information for practitioner and gastroenterologist alike. Written with an ease of style possible only for those who have attained mastery of their subject, it becomes a piece of medical literature.

The volume is rather comprehensive for textbook purposes. It should probably find its greatest value as a book of reference for practitioners and students. On the other hand, the gastroenterologist's library is incomplete without it.

The author is to be commended upon the way in which he presents his subject matter. His approach to the main theme is logical and sequential. The work is divided into three parts as follows: Part I contains chapters on anatomy, embryology, physiology, laboratory methods, x-ray, and gastroscopy. Part II deals with the diseases of the stomach and Part III is for the most part taken up with a consideration of the effect of gastric diseases upon other important organs. The work abounds in helpful illustrations, charts, diet lists, and regimens.

Principles of Pathology†

THE task confronting the writer of textbooks is becoming increasingly difficult. He must determine, first, to which audience his effort shall be addressed, the student, the practitioner, or the specialist, and, second, whether he shall endeavor to present all the minutiae of the subject, or only those phases of it which may be described with certitude.

And, in the background, there is always the possibility that much of what he has written laboriously and after painstaking labor, may become obsolete through the advances made in the subject.

Power and Hala have selected the student and the practitioner as their audience and have written their book both for those who approach the subject for the first time or who desire to refresh their memories "on points long forgotten, or in regard to recent discoveries."

They have endeavored, therefore, to prepare a concise text in language not overburdened with exuberant nomenclature and understandable to the novice.

In this undertaking they have been eminently successful. As said in the preface: "There is very little that cannot be said in plain English and that is how we prefer to say it."

Very wisely, there is no attempt to make the book encyclopedic, the authors concentrating rather on what is essential and important leaving the rare and unusual to monographs.

The book may be warmly recommended not only to the student and practitioner but to all who desire a succinct presentation of the basic principles of pathology sometimes overlooked when "the forest cannot be seen for the trees."

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*Diagnosis and Treatment of Diseases of the Stomach with an Introduction to Practical Gastro-Enterology. By Martin E. Rehfuss, M.D., Assistant Professor of Medicine in Jefferson Medical College. Reprinted one month after publication. Octavo of 1236 pages with 519 illustrations, some in colors. Cloth. W. B. Saunders Company, Philadelphia, Pa.

†Principles of Pathology for Practitioners and Students. By H. d'Arcy Power, Professor of Pathology, College of Physicians and Surgeons, San Francisco and William W. Hala, Assistant Professor of Pathology, Long Island College Hospital, Brooklyn, Cloth, 787 pages, 298 illustrations, 12 in color.

NOTE: In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

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EDITORIALS

The Bacteriology of Genitourinary Tract Infections

NONSPECIFIC infections of the genitourinary tract in the vast majority of cases are usually regarded as due to B. coli if pus cells and gram-negative bacilli of conformable morphology are found in the urine. It is seldom that extensive bacteriologic studies are carried out in confirmation of the diagnosis and still less frequent are reports of studies of the bacterial flora of the genitourinary tract, although the presence of adventitious bacteria is often a complicating factor in the determination of the etiology of a pyelitis, for example.

Recent studies along this line, therefore, are of some interest and possess, perhaps, some clinical application warranting a survey of the data so far obtained.

A study of two hundred cultures of gram-negative bacilli isolated from two hundred cases of genitourinary infection has recently been presented by

Hill, Seidman, Stadnichenko, and Ellis[1] representing an endeavor to determine the bacteriologic characteristics of the organisms and their possible clinical correlation.

The study is complicated not only by the constant presence of adventitious organisms but also by the multiplicity of bacteria somewhat loosely classified as belonging to the colon group.

To avoid the former difficulty, before the collection of the specimen, these workers first cleansed the urinary meatus with alcohol and the anterior urethra by an injection of 1:500 meroxyl.

The second factor necessitated an extensive and varied bacteriologic study, the results of which form the subject of the report.

These workers are convinced, first of all, that "there must be a careful correlation of thorough examinations of direct smears with cultural results if accurate bacteriologic findings are to be obtained."

Rarely, in cases undergoing intensive treatment, organisms may be absent in the smear and recovered by culture but, in general, "an organism which develops scantily on culture, usually only after forty-eight hours of incubation, and which has not been seen in the direct smear, can be discarded as a urethral contamination."

The cultures studied in this series are divided for convenience into four groups:

I. One hundred Escherichia cultures, fermenting lactose with acid and gas, not producing acetyl-methyl-carbinol, but methyl-red positive.

II. Seventy-nine Aerobacter cultures, fermenting lactose with acid and gas, producing acetyl-methyl-carbinol, but methyl-red negative.

III. Five Proteus cultures.

IV. Sixteen miscellaneous cultures.

Attention is called to the differential value of citrate media which the Aerobacter forms utilize promptly and the Escherichia belatedly, scantily, or not at all.

The most significant facts derived from the correlation of bacterial groups with the different types of clinical infections encountered are thus summarized:

1. Seventy-five per cent of the blood stream invasions were due to organisms of Group II (Aerobacter) in the 12 cases in which the same organism was recovered from the blood and urine, the incidence of Group I (Escherichia) being only 1, or 8.3 per cent. This high proportion of Aerobacter cultures in such blood stream infections parallels the high incidence of this group of cultures in genitourinary infections, as compared with organisms present in the intestinal flora.

This observation suggesting a differentiation of the colon group in blood stream invasions is, apparently, new and suggests the necessity of further studies along this line.

2. In 25 cases of lithiasis, Group II (Aerobacter) organisms were encountered in 48 per cent, and proteus in 12 per cent, the remainder (40 per cent) being Group I (Escherichia).

3. In three cases of abscess formation, two developed blood stream invasions.

In a further study Stadnichenko[2] reports upon thirty strains of gram-positive cocci isolated from genitourinary infections, the most prevalent type of infection being prostatitis, although there was no correlation between the type of infection and the organisms isolated.

In fifty cases bacteriologically studied Gondolf and Stringer[3] found that over half the cases proved to be organisms other than the Escherichia group.

The results thus summarized cast grave doubt upon the efficiency of the stock B. coli vaccines not infrequently resorted to in the treatment of this type of infection.

Finally, Scudder and Belding[4] report upon the characteristics of a group of higher bacteria from the genitourinary tract belonging, apparently, among what used to be classified as cladothrix or streptothrix, which may be mistaken for streptococci, and which have, perhaps, some relation to chronic infections in the urinary tract.

It would appear that this subject is worthy of extensive and, of necessity, prolonged study.

REFERENCES

1. Hill, J. H., Seidman, L. R., Stadnichenko, A. M. S., and Ellis, M. G.: A Study of Two Hundred Cultures of Gram-Negative Bacilli Isolated From Cases of Genito-urinary Infection, J. Bacteriol. **17:** 205, 1929.
2. Stadnichenko, A. M. S.: Thirty Strains of Gram-Positive Cocci Isolated From Cases of Genitourinary Infection, J. Bacteriol. **17:** 303, 1929.
3. Gondolf, H. J., and Stringer, M. S.: A Bacteriologic Study of the Gram-Negative Bacilli Found in Fifty Cases of Infection of the Urinary Tract, New Orleans Med. & Surg. J. **81:** 735, 1929.
4. Scudder, S. A., and Belding, D. L. A. Group of Higher Bacteria From the Genitourinary Tract, J. Lab. & Clin. Med. **14:** 801, 1929.

—*R. A. K.*

What Is an Internist?

THE term, Internal Medicine, has come within recent years into almost universal usage. And yet few terms appear to be more difficult to define. A medical organization of national prominence has in the past had as a part of its program the education of the lay public in the significance of this term. The directory of The American Medical Association classifies internist or internal medicine as a separate and distinct specialty. And yet how many can formulate a concise definition of the term.

The present writer having made the attempt and failed, undertook to obtain a series of definitions or expressions of opinion from a group of pre-eminent so-called internists throughout the country. Questionnaires were also sent to a few men in specialties other than internal medicine. The results are sufficiently interesting to bear analysis.

Those replying may be classified roughly into three groups: first, men whose primary interest is the teaching of medicine in our leading medical schools; second, those primarily interested in the practice of internal medicine. The viewpoint of this second group might be expected to differ slightly from the first inasmuch as members of the second have, theoretically at least,

had more contact with borderline cases, diseases that might be treated in any one of several specialties such as dermatology, neurology, and pediatrics. The third classification includes men practicing these other specialties who might be expected to voice the criticism that the internist trespasses upon their field of work.

Twenty-three definitions or descriptions are at hand. As might have been anticipated, the classifications of the replies bear no relationship to the three groups mentioned above. Nearly all agree on the great difficulty of defining an internist or internal medicine and many prefer to submit a description rather than a definition.

GROUP I

Three offered interpretations based on the literal significance of the term, relating it to diseases of the internal organs. They are as follows:

1. "Until I received your letter I had never tried to define internist or internal medicine. Had anyone asked me casually what was meant by these words I should have given him at once a definition. Now when I try to put it in writing I find the thing is not as easy as I thought. However, I should like to submit the following:

"An internist concerns himself with the diagnosis and treatment of diseases having their seat in internal structures and tissues of the body. He excludes from his field the diseases peculiar to the organs of special sense, the diseases of infancy, and those methods of treatment that belong to the category of surgical operations.

"Internal medicine is a branch of medicine that concerns itself with the diagnosis of diseases of the internal organs and tissues of the body, and with their treatment in so far as such treatment does not involve the use of surgical procedures and methods that belong to the well-defined specialties of medicine."

2. "I notice that the Dorland's American Illustrated Dictionary defines an internist as a physician who treats diseases of the internal organs. This, I think, is not a very good definition unless one qualifies it by saying exclusive of the organs of generation. If this qualification is not made then the internist becomes an obstetrician and gynecologist. It may be added that such a definition is not sufficiently inclusive because an internist also treats peripheral neuritis, vascular diseases of the limbs, etc., which are certainly not internal organs. The reason why it is so difficult to write a concise definition is that the work of an internist is not limited by anatomical or physiologic considerations but by custom. I doubt whether any definition of an internist can be formulated briefly. Of course one might go further and say that an internist should not treat psychiatric or neurologic conditions. The more I think of it, it is impossible to draw any hard and fast line as to just what an internist should cover."

3. "I have delayed answering your letter about a definition of the expression, 'internal medicine' and 'internist,' because I have found this so hard to do. How would this do?

"Internal medicine is concerned with everything which influences the general health or nervous stability of the patient. It is particularly concerned with disorders of the organs embraced in the thorax and the abdominal cavity."

It is impossible in the above group as well as in those that will follow to limit any one reply to a single group, for many of them include features of two or more of these classifications.

GROUP II

The next group roughly comprises those who, instead of *defining* internal medicine and an internist, *describe* them, chiefly in terms of the limitations of the field with respect to the other specialties.

4. "Internal medicine is a branch of the practice of medicine that has to do with the diagnosis of medical and surgical diseases and with the treatment of medical diseases which are not entirely within the scope of the more highly specialized branches of medicine such as dermatology, neurology, and pediatrics."

5. "I should define an internist as one who concerns himself with the diagnosis and the nonsurgical treatment of diseases involving any organ or tissue, in the adolescent or adult human body, except the skin, the nose and throat and the special senses.

"Internal medicine is concerned with the same subjects. Obstetrics is excluded because childbirth is not a disease. The toxic, diseased conditions associated with it, however (eclampsia, anemia, vomiting), are within internal medicine."

6. "I think it is very hard to define internal medicine or an internist. Either can be described better than defined. I have always looked on internal medicine as being that part of medicine which was left over after operative surgery, obstetrics, and the specialties which require technical procedures in diagnosis or treatment have been taken away. This is certainly not a definition and probably a poor description."

7. "Internal medicine is medicine minus surgery and the specialties.

"An internist is one who devotes himself intensively to internal medicine and thus becomes a specialist in internal medicine."

8. "By an internist I mean a man who practices internal medicine as distinguished from the various specialties, which he should, of course, leave severely alone.

"The two fields on which he could encroach to some extent would be pediatrics and neurology. As an internist I feel at perfect liberty to take care of children over two years of age, either at my office or at their homes. I do think that infants are out of our field. Neurology has always been closely allied to internal medicine, and is included in all the textbooks on the subject. My rule here would be to call on a neurologist when I was up a tree.

"The various subdivisions of internal medicine are now becoming specialties, but that does not preclude their being handled by the internist. I refer, of course, to cardiology, gastroenterology, metabolism, tuberculosis, syphilis and the diseases of allergy. I use specialists on these subjects for convenience. In other words, if I am perplexed over a case, I refer it to one of them for diagnosis and possibly treatment. As long as I feel equal to the situation, I keep the patient under my own care.

"I do not believe a real internist should ever touch a scalpel, nor do I believe that he should handle conditions in the nose or throat or genitourinary tract.

"Some of the skin diseases are so dependent on internal medicine that they could better be handled by the internist than by the dermatologist. I refer particularly to the allergic conditions."

One correspondent whose definition appears under Group IV offered the following remarks relative to this method of defining internal medicine by describing its limitations:

9. "It seems to me that the so-called medical specialties inclusive of neurology, gastroenterology, cardiology, hematology, pediatrics, and genitourinary medicine (aside from surgical therapy) all belong in internal medicine.

"Obstetrics is of course excluded from internal medicine.

"The suggestion that internal medicine is distinguished from the various specialties and that the internist should leave the specialties severely alone could not, in my opinion, be adopted, for if it were, the internist would have nothing to do since practically every part of internal medicine is included in one or another specialty.

"Instruments of precision are used by internists as well as by all specialists in medicine and in surgery for diagnostic purposes. The ophthalmoscope for example is a necessary instrument in the work of the internist.

"Moreover, laboratory methods must be applied by all medical practitioners, internists, surgeons, and specialists.

"The internist must also be able to make diagnoses in diseases that require surgical treatment, for example, appendicitis, cholecystitis, Banti's disease, nephrolithiasis, brain tumor, etc."

GROUP III

Roughly the replies in this group express the opinion that internal medicine cannot be closely defined as a specialty but includes a very broad territory, taking in portions of many of the specialties and yet at the same time being distinct from the field covered by the so-called general practitioner. There is no attempt at rigid delineation of the internist's field of activities.

10. "I think that Dr. Osler was one of the first in this country, possibly the first, to employ the term, internal medicine. I quote from the first paragraph of his essay on Internal Medicine as a Vocation, published in the volume entitled *Aequanimitas*. He says:

"'I wish there were another term to designate the wide field of medical practice which remains after the separation of surgery, midwifery, and gynecology. Not itself a specialty (though it embraces at least half a dozen), its cultivators cannot be called specialists, but bear without reproach the good old name physician, in contradistinction to general practitioners, surgeons, obstetricians, and gynecologists.'

"The term originated in Germany where it is used synonymously with clinical medicine. I have always employed the term in the sense in which it was used by Dr. Osler."

11. "I certainly do not differ from you in finding it difficult to define an internist. Of course he is one who is concerned only with nonsurgical diseases, and it has been my understanding that the term has been derived from the fact that most of the diseases which come to the hands of the internist are of the internal viscera, and to employ these of broader significance, usually systemic in character. I should say that the term expressed exactly the opposite of the term general surgeon."

12. "According to my understanding *internist* is nearly synonymous with *physician*. General practitioner is more inclusive, for he, like the country doctor, will usually undertake to do at least minor surgery. As a matter of fact the old term *physician* seems to me to have especially connoted doctors whose chief employment was the *treatment* of disease by any methods, including those of minor surgery; he was a 'general practitioner.'

"The term internist seems to have come into vogue since the development of *laboratory medicine* as a distinct specialty.

"Laboratory methods have tended to exalt *diagnosis* and to more or less segregate it as a specialty based on exact explorations.

"A new branch of medical science and art is represented in the technic, and its interpretative judgment, developed in the clinical laboratory.

"Physicians trained to appreciate this new source of information naturally turn to it constantly, not only for suggestive and confirmatory diagnosis but for crucial evidence of the effects of *treatment*.

"With this increase of exactness in the appreciation of pathologic complexes the physician has naturally more and more eschewed the use of things and methods with which he is not intimately conversant: He seeks to be a specialist in any task he undertakes; he therefore lops off surgery from his practice in toto. He confines himself to that systematization of phenomenon which we know as diagnosis, and he selects therefrom for experimental study (treatment), such complexes as have for him particular interest.

"He is, I take it, an *internist*. Woe is you! Boil this down to a sentence."

13. "It has always seemed to me that internal medicine was a very incorrect way of designating that branch of medicine which includes everything not surgical. So, too, the designation of the man practicing nonsurgical medicine as an internist.

"The more one thinks of the type of work the nonsurgical practicing physician does in his daily routine, the more difficult becomes a concrete, concise definition thereof.

"The nonsurgical men in their diagnostic work, if they are up to the minute in the progress of their profession, are called upon to do and try to accomplish diagnosis in nearly the entire field of medicine, even invading the ophthalmologic area. Nevertheless in their clinical work, or in the field of therapeutics they studiously avoid all of those disorders which call for the application of surgical methods."

14. "The question embodied in your letter is certainly a facer. After giving the question due consideration, here goes my definition.

"Internal medicine concerns itself with the clinical pathology of the so-called general diseases, but should include a thorough grounding in the specialties.

"An internist is one who concerns himself with the diagnosis and treatment of general medical conditions, but to profess such he should be thoroughly grounded in all the special fields of medicine."

15. "My ideas regarding specialism in medicine are quite old-fashioned. I believe that every medical man should have a thorough general medical education, and that he should practice general medicine for some time before he ventures upon a specialty. This I believe to be as important in the case of internists as in the case of a neurologist or psychiatrist.

"I cannot give you an accurate definition of an internist but he is or should represent in his activities general medicine. He should be a man who is familiar not only with visceral diseases and the general affections to which the organism is subject, but also should have a reasonable knowledge of diseases of the eye, ear, skin, and of the brain and spinal cord, besides an acquaintance with the problems of psychiatry. While he cannot of course be a 'universal specialist,' these qualities which I have mentioned are in my judgment eminently desirable."

16. "Though internal medicine is extensively used, it is interesting that so far as I know no chair in any medical school in the United States carries the defining adjective 'internal' but instead 'medicine' is used with, in some of the old medical schools the use of the term 'physic' instead of 'medicine.' I have an idea that we would have done best if we had held to this terminology and used medicine rather than internal medicine."

GROUP IV

In this group the idea is emphasized that an internist by virtue of special training and experience has developed a broader perspective and is therefore capable of clearer insight than his fellow practitioner. The first two contributions in this group are perhaps two of the most successful attempts at a brief, concise definition. More exception could be found for the first than for the second. Here as elsewhere throughout these classifications definitions which are included in other groups might be included in part at least within this group.

17. "Internal medicine is the art and science of correctly interpreting the individual's maladjustment to environment.

"An internist is a physician who, through skill in these arts and sciences, is able to understand the individual and correct the maladjustment."

18. "A physician skilled in diagnostic methods, possessing a comprehensive understanding of the etiology and pathology of disease, with a knowledge of psychology, pharmacology, immunology and pathologic physiology, adequate to enable him to prescribe a rational therapy."

19. "My inference is that the internist must base his practice on a primary, intimate knowledge of pathology, on an accurate and extensive schooling in all the methods of diagnosis, and must be schooled in all therapeutic measurements except those involving surgery."

20. "In the clinics of Germany and Austria the professor of medicine, as distinguished from surgery, has headed the department known as 'Innere Medicin.' The patients oc-

cupying his wards were believed to have 'innere krankheiten' or internal diseases requiring study of the function or disability of internal organs. I believe the term 'innere medicin' originated in the application of more refined methods of diagnosis before treatment, that is, the methods were carried further than was possible in other departments of the hospital or by the so-called general practitioner of medicine who covered the whole ground, more or less, outside the hospital. The term internal medicine was the translation of Innere Medicin and as such has come into general use by physicians. It is interesting to note that the Standard Dictionary does not define internal medicine or internist. Dorland's American Medical Dictionary defines an internist as 'a physician who treats diseases of the internal organs' and internal medicine as 'that department which deals with diseases that cannot be treated surgically; medicine as distinguished from surgery.'

"An internist, as I understand the term, is one who has had special training in the refinements of diagnosis, and whose judgment has been founded upon extended experience in general diseases not requiring surgical relief. I acknowledge that the term internal medicine is difficult to define in a few words, and that it is confusing to many Americans. The field which it covers is fairly well understood on the European Continent. I have no suggestions as to better terminology."

GROUP V

In this last group a new factor appears. This is the conception of the internist as an integrator of all the facts discovered or discoverable in an individual case not only by himself but also with the help of any specialists, and the director of their proper application. This is the concept that is put to practical application in the proper working of group medicine.

21. "I share with you the difficulty of finding a satisfactory definition of an internist. Of course the term was introduced to distinguish the worker in internal medicine from the worker in so-called external medicine. The former included the study of the body outside of the provinces that belonged to surgery whereas the latter included surgery, the surgical specialties and perhaps dermatology.

"Of course such a definition no longer holds, for the internist has adopted the biologic view of the organism that views each patient as a phenotype or realized person that has resulted from a succession of interactions of environment with the genotype. As a result of this biologic conception of the organism as a whole the internist has become the leader in general diagnostic studies in which complete analyses of all the systems of the body are made, after which the findings are synthesized into a multidimensional diagnosis with the various indications for therapy that this study reveals. As a rule internists, therefore, make much more careful and much more comprehensive studies of the organism and all its parts than do surgeons or the surgical specialists or even the so-called specialists in medical domains (gastroenterology, cardiology, neurology, etc.).

"It seems to me that this recent development is a movement in the right direction and that probably all patients should be subjected to a general diagnostic study, for often the domain in which their complaints seem to be centered is not the domain in which the main trouble exists. There is no reason, of course, why a surgeon or a specialist should not conduct such general diagnostic surveys and make such multidimensional diagnoses if his training and experience fit him for it, but on the whole internists are, as a rule, better fitted by training and experience for the conduct of such general diagnostic surveys with subsequent integration."

22. "There should not be any such thing as 'internal medicine' because I think it is impossible to define. However, after laboriously considering the matter, I submit the following, unsatisfactory as it is:

"Internal medicine—the branch of medical practice characterized in distinctive measure by its aim at definitive treatment, advised by the practitioner in this branch, to be carried out by himself or others more specially expert, according to the patient's need, as re-

vealed by a relatively comprehensive diagnosis made by the practitioner in this branch, as a result of his correlation of his investigations, supplemented by those of any specially qualified consultants who may have been selected by him.''

23. ''An internist is a physician who limits his work to diagnosis and consultation. Filling, as he does, a place which overlaps every other department of medicine, he correlates the work of other men and can sit in judgment on all procedures which are not technically surgical. He is essentially a helper and can treat on request.

''When he treats cases which come to him of *their own volition*, he is merely a practitioner of medicine, with such deletions of patients as he individually debars.''

24. ''Concerning the definition of internal medicine and of an internist, I would say that you have asked a good one. I would find it difficult or impossible to express it in a few words. An internist, in my mind, is a physician who concerns himself with the diagnosis, prognosis, and treatment of all adults, no matter what the complaint. I make that distinction in order to permit a subdivision of pediatries. The treatment of some of these conditions that he will diagnose will necessarily be delegated to other specially trained physicians. Having found out that the patient has a brain tumor, he is naturally sent to a competent neurologic surgeon. Having diagnosed the abdominal tumor as due to pregnancy, that patient goes to an obstetrician. Having found that the itch that troubles the patient is due to pityriasis rosea, he probably will be sent to a dermatologist. But if it is due to Hodgkin's disease, he will probably look after it himself with the aid of the roentgenologist, etc. He may therefore treat a remaining group of patients that he feels competent to care for, and even here he may again delegate part of them to special internists because of particular qualifications that they may have.''

From a review of the preceding expressions of opinion on the significance of the terms Internist and Internal Medicine, the difficulties of concise definition become obvious. Indeed, we are inclined to agree with those correspondents who feel that it is impossible to define internal medicine because it blends in to so many of the specialties.

After reviewing the above definitions contributed by leading thinkers throughout the country one would have considerable temerity in proposing a substitute definition. Internal medicine is in the last analysis a misnomer and therefore any attempt to define it must be to some extent patch work. The field of internal medicine does not deal exclusively with diseases of the internal organs although to be sure this may be said to be its major interest. Furthermore, other specialties such as urology, gynecology, proctology, roentgenology also deal with diseases of the internal organs.

There can be no specific definitive limitation with regard to the other specialties and it is readily understandable how different definers will disagree as to what of the medical specialties may be encroached upon. One will include neurology as a subdivision of internal medicine and another will exclude it. The limitations of internal medicine vary in the different diseases and even with individual cases. Iritis when due to local infection should properly be treated by the ophthalmologist but when it is syphilitic in origin its treatment falls within the domain of internal medicine. Peptic ulcer and cholangeitis are not necessarily always surgical. One case will be treated medically, another surgically, even by the same physician. The internist may refer one case of dermatitis to a dermatologist while he may prefer to treat his own case of arsenical dermatitis and his own allergic dermatitis. The treatment of diabetes in children does not differ from that of adults.

It becomes apparent therefore that internal medicine cannot be defined or described precisely in terms of the extent and limitations of its domain.

By tacit consent it would appear that the term internist implies an individual with an unusually broad perspective, certainly broader than that of the more highly specialized practitioners, and possibly a keener insight into the problems of the patient, considered in terms of his entire economy as a unit, and its relationship to his environment. The internist views the patient as a whole and modifies his treatment for a specific ailment in accordance with his understanding of his patient as an economic entity.

As a consequence the terms integrator, correlator, advisor, consultant and director may properly be applied to some of the functions of the internist. On him falls the burden for deciding for or against therapy in other special fields such as surgery, the decision being reached by virtue of intimate knowledge of all the factors which might have a possible bearing on the results of such treatment. Among such factors are the presence of cardiovascular or renal pathology, tuberculosis, diabetes, mental instability and indeed, not infrequently the patient's domestic problems and even his financial status.

In the field of diagnosis the internist must be a man of wide vision and must keep himself as free as possible from pet theories and hobbies. In the field of therapy he must retain for himself considerable latitude as to what he shall elect to treat himself. A patient with arthritis is referred to an internist for study and whatever treatment may be necessary. During the course of search for focal infection a crowned tooth is observed, infected tonsils are recognized and pus is obtained from the prostate by massage. In this one case, the internist at once proves himself inconsistent. He sends his patient to the dental radiologist for x-ray of the tooth and to an exodontist for tooth extraction. He refers him to an otolaryngologist for tonsillectomy. By the rules of the game he should refer him once more to a urologist for prostatic masssage and bladder irrigation. But he considers himself entirely competent to perform this therapeutic procedure and proceeds to do so. If the patient be a woman with an endocervicitis, he may send her to a gynecologist, or, if proficient, he may himself cauterize the cervix thus clearing up this focus of infection. Provided he is competent in the fields of therapy which he may undertake, the internist reserves for himself considerable latitude.

It is true that internal medicine overlaps nearly all of the specialties. This is an argument in favor of saying that internal medicine is not in itself a specialty. It is equally true that many of the specialties overlap, not only internal medicine, but likewise other specialties. The diseases are numerous in which a patient will wander from one specialist to another, finally finding the one who can give most relief. And with the same disease it is not always the same type of specialist who accomplishes best results in every case. Syphilis is treated by the dermatologist and by the internist. The toxemias of pregnancy are treated by the obstetrician and by the internist. Arthritis comes within the domain both of internal medicine and of orthopedics. Malignancy is treated by the surgeon. It is also treated by the

roentgenologist. The same is true of fibroid tumors. Examples might be multiplied.

One very competent pediatrist always insisted that he was not a specialist but was a general practitioner among children.

The anteroom of Sir Jonathan Hutchinson, one of the leading surgeons of his time, was filled at all times not only with surgical cases but with skin conditions, individuals with cardiovascular diseases, and in short the entire gamut of the medical diseases. Sir Jonathan, once questioned humorously as to his definition of surgery, with a twinkle in his eye slapped his hand upon the table saying, "Surgery is anything that comes to Sir Jonathan Hutchinson." Possibly the average internist feels much the same way about internal medicine. It has been said that an internist is just a glorified general practitioner. Here is food for thought.

The young physicians recently graduated from our leading medical schools, after completing their terms of internship, unless they have undertaken some one of the other specialties, designate themselves as internists. Indeed, the ideal to be aimed at in medical education is to give each student such thorough undergraduate and postgraduate instruction that every practitioner will be in essence an internist. This brings us back to the concept expressed in several of our contributions, that the good term physician is more appropriate and more descriptive than the term internist.

Internist and Internal Medicine are misnomers. Are there no better, more appropriate designations which may be used?

Physician has been suggested. There is another term which has gained great popularity with the laity, but which is not in such good grace with the medical profession. This is the term Diagnostician. In its more common implication, it is a designation not to be desired. The average layman thinks of a diagnostician as an individual who can give you a name for your malady, ticketing you, labeling you, but who is little interested in therapy. This lay understanding of the term has come about as a result the use of the so-called diagnostician as a consultant. After the consultation the attending physician rather than the consultant proceeds with treatment.

Even within the profession this understanding of the term has been rather widely accepted. Dorland's Dictionary which derives the word diagnosis from two Greek words meaning "to know apart," defines diagnosis as the art of distinguishing one disease from another. In this sense diagnosis would be but the classification of diseases.

But if we will go back to the original Galenic interpretation of the term, we will find that it assumes decidedly deeper significance. True, *dia* does mean "apart" but it has other translations. It may be translated as "through" or "throughout" and indeed is so translated in connection with other words in Dorland's Dictionary. In this sense diagnosis would mean *"to know through or throughout or thoroughly, to understand thoroughly."* When Galen said that it took him long to diagnose the pulse, that it does not merely rise and fall but expands and contracts, he meant that it took him a long time to thoroughly understand the pulse.

In this deeper sense Diagnosis and Diagnostician should find better favor. A diagnostician would be one with a thorough understanding of the patient's malady. This understanding would apply not only to *classification* but also to *treatment*. One cannot thoroughly understand a malady unless at the same time one knows the best remedies to apply and how to apply them.

The writer is not suggesting that the term Diagnostician be substituted for that of Internist, although aside from its recognized shortcomings it appears to be a more appropriate term.

But his understanding of the term diagnosis impels him to venture yet another definition of an internist. The following definition is not submitted as a composite of the twenty odd definitions recorded above nor as a substitute but merely as yet one more concept which it is hoped may stir up productive discussion on the subject. No claim for entire originality can be made since the same idea is expressed in several of the definitions which have been contributed above.

The points submitted in favor of this definition are: (1) that it avoids some of the apparently weak points in certain of the definitions, such as relationship to internal organs, and the attempt to define the limitations of internal medicine in terms of the specialties; (2) that it includes the concept of an internist as being first of all a physician; (3) and the idea of correlation; and (4) that it is relatively brief and concise. True, it deals in generalities, but by now we must agree that the word is only susceptible to definition in general terms.

25. "An internist is a physician who through adequate training and experience has reached that stage in the art and science of medical practice, at which he possesses as thorough an understanding as possible of the nature of the maladies from which his patients are suffering; and is competent to prescribe appropriate treatment, or to advise the proper form of additional study or treatment, to be administered by another practitioner in some specialized field of medicine."

—*W. T. V.*

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Dr. Frederic E. Sondern, New York, N. Y. 1925-6
Dr. Wm. G. Exton, Newark, N. J. 1926-7
Dr. A. H. Sanford, Rochester, Minn. 1927-8
Dr. F. W. Hartman, Detroit, Mich. 1928-9

News and Notes

We read in *Science* that Dr. John A. Kolmer, professor of pathology and bacteriology in the graduate school of medicine of the University of Pennsylvania, was recently awarded the Mendel medal by Villa Nova College for his work in immunology. This is the first award of the medal, which was established to commemorate the work of Gregor Mendel.

Dr. Benjamin S. Kline of Cleveland will officially represent the American Society of Clinical Pathologists at the dedication of the new building of the Institute of Pathology of the Western Reserve University of Cleveland, Ohio.

The Committee appointed to look after local arrangements for the 1930 Convention of the American Society of Clinical Pathologists in Detroit, Michigan, are as follows: Dr. Frank W. Hartman, Chairman, Dr. Arthur L. Amolsch, Dr. O. A. Brines, Dr. H. E. Cope, Dr. O. M. Gruzhit, Dr. Walter E. King, Dr. Clarence I. Owen, Dr. R. G. Owen and Dr. C. M. Stafford, all of Detroit.

Dr. D. Schuyler Pulford, Woodland Clinic, Woodland, California, has been appointed by our President, Dr. J. H. Black, to serve on the Program Committee in the place of Dr. Wm. G. Exton, Newark, N. J.

A Columnist's Conception of a Technician

The morning mail brings a letter from a gentlemen whose stationery proclaims him as a "Microscopist, Technologist, and Scientist." He asks for assistance in hunting a job. And him with a microscope!

The Office of the Society is in receipt of numerous invitations from cities throughout the country who are anxious to have our Convention in their midst. From one of these a

letter is addressed to the American Society of CRIMINAL Pathologists. Comment: We wonder whether this is an attribute of the profession or refers to the practice of forensic medicine.

Letter From S. C. Dyke, D.M., Wolverhampton & Staffordshire Hospital, Secretary of British Pathologists' Association

Wolverhampton, 2/9/29.

Dear Dr. Corper:

Thank you for your letter of August 8th. I take it as a great honor both to myself and to the British Pathologists' Association that your Society has seen fit to elect me a Corresponding Member. Will you be so good as to convey to your Society the thanks of my Association?

I will see that copies of all transactions of my Association are forwarded to you and that you are kept posted as to any change in the secretaryship.

I shall take the first opportunity of bringing your letter before the general body of my Association.

I am

Yours very sincerely,

(Signed) S. C. DYKE.